AUDITION
## CONTENTS

Abstracts are grouped by Subject Categories in alphabetical order by first author.
* Indicates nonmember of the Society for Neuroscience.

<table>
<thead>
<tr>
<th>Subject Category</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Audition</td>
<td>1</td>
</tr>
<tr>
<td>Autonomic Function</td>
<td>15</td>
</tr>
<tr>
<td>Axonal Transport</td>
<td>29</td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td>39</td>
</tr>
<tr>
<td>Brain Metabolism and Nutrition</td>
<td>53</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>61</td>
</tr>
<tr>
<td>Cerebral Cortex</td>
<td>71</td>
</tr>
<tr>
<td>Chemical Senses</td>
<td>83</td>
</tr>
<tr>
<td>Comparative Neurobiology</td>
<td>95</td>
</tr>
<tr>
<td>Development and Aging</td>
<td>105</td>
</tr>
<tr>
<td>Drugs of Abuse</td>
<td>131</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>137</td>
</tr>
<tr>
<td>Evoked Potentials and EEG</td>
<td>149</td>
</tr>
<tr>
<td>Extraocular Movement</td>
<td>159</td>
</tr>
<tr>
<td>Feeding and Drinking</td>
<td>169</td>
</tr>
<tr>
<td>Invertebrate Neurobiology</td>
<td>185</td>
</tr>
<tr>
<td>Limbic System</td>
<td>213</td>
</tr>
<tr>
<td>Membrane Biophysics</td>
<td>231</td>
</tr>
<tr>
<td>Membrane Structure and Function</td>
<td>241</td>
</tr>
<tr>
<td>Memory and Learning</td>
<td>253</td>
</tr>
<tr>
<td>Monoaminergic Systems</td>
<td>265</td>
</tr>
<tr>
<td>Motor Systems</td>
<td>289</td>
</tr>
<tr>
<td>Neurochemistry</td>
<td>309</td>
</tr>
<tr>
<td>Neurocytology</td>
<td>327</td>
</tr>
<tr>
<td>Neuroendocrinology</td>
<td>337</td>
</tr>
<tr>
<td>Neuroethology</td>
<td>359</td>
</tr>
<tr>
<td>Neuromuscular Junction</td>
<td>365</td>
</tr>
<tr>
<td>Neuronal Circuits and Pattern Generation</td>
<td>377</td>
</tr>
<tr>
<td>Neuronal Shape and Function</td>
<td>385</td>
</tr>
<tr>
<td>Neuropathology and Neuroimmunology</td>
<td>393</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>403</td>
</tr>
<tr>
<td>Neuropharmacology</td>
<td>417</td>
</tr>
<tr>
<td>Neurotransmitters</td>
<td>439</td>
</tr>
<tr>
<td>Pain</td>
<td>455</td>
</tr>
<tr>
<td>Plasticity</td>
<td>465</td>
</tr>
<tr>
<td>Psychopharmacology</td>
<td>483</td>
</tr>
<tr>
<td>Receptors</td>
<td>507</td>
</tr>
<tr>
<td>Regeneration</td>
<td>527</td>
</tr>
<tr>
<td>Sleep</td>
<td>537</td>
</tr>
<tr>
<td>Somatosensory Systems</td>
<td>545</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>561</td>
</tr>
<tr>
<td>Synaptic Transmission</td>
<td>575</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>587</td>
</tr>
<tr>
<td>Trophic Functions</td>
<td>599</td>
</tr>
<tr>
<td>Vestibular System</td>
<td>607</td>
</tr>
<tr>
<td>Vision</td>
<td>617</td>
</tr>
</tbody>
</table>

Indexes

<table>
<thead>
<tr>
<th>Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author Index</td>
<td>653</td>
</tr>
<tr>
<td>Topic Word Index</td>
<td>675</td>
</tr>
</tbody>
</table>
1 MORPHOLOGY AND PHYSIOLOGY IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS. JOE C. ADAMS LMQ, NINCDS, NIH, BETHESDA, MD 20014

In the ventral portion of the ventral nucleus of the lateral lemniscus (VRL) 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 VRL are similar to those in the cochlear nucleus.


Numerous parallel neural pathways exist in the central auditory system of mammals. In the hindbrain alone, the cochlear nucleus projects to the inferior colliculus (1directly. 2via the ipsilateral superior olivary complex —> contralateral inferior colliculus pathway. 3via the contralateral medial nucleus of the trapezoid body —> superior olivary complex —> inferior colliculus pathway. 4via 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.

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

The responses of 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; BANDS 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 recordings in 6 unanesthetized cats. These results demonstrate two parallel pathways from the inferior colliculus to medial geniculate, one originating in 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 pericentral nucleus, only 23% 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 ventrolateral 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 ventrolateral division. 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 pattern to at least the ipsilateral anterior auditory field (AAF). These experiments indicate that reciprocal connectivity and the vertical organization of connections within bands are important rules of structure for the auditory cortex.

*Indicates nonmember of the Society for Neuroscience.

(Supported by NIH Grant NS-05046.)

(Supported by NIH postdoctoral fellowship NS-05046.)
DEPENDENCE OF INTRACELLULAR RESPONSES ON SOUND STIMULUS PARAMETERS IN HAIR CELLS AND SUPPORTING CELLS OF THE ALLIGATOR LIZARD COCHLEA. Keld Boden-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). 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 symmetrical 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. Fundamental components of the response to tones was measured as a function of frequency for constant sound-pressure levels at the tympanic membrane. These frequency-response curves were relatively broadly tuned, often exhibiting two best frequencies.

PARAMETERS IN HAIR CELLS AND SUPPORTING CELLS OF THE ALLIGATOR LIZARD COCHLEA. Keld Boden-Kristensen* and Thomas F. Weiss. Eaton-Peabody Laboratory of Auditory Physiology, Massachusetts Eye and Ear Infirmary, Boston, MA 02114.

The responses of hair cells were independent of repetition rate from 10 to 150 clicks/sec. At low click levels, response waveforms were oscillatory and symmetrical 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. Fundamental components of the response to tones was measured as a function of frequency for constant sound-pressure levels at the tympanic membrane. These frequency-response curves were relatively broadly tuned, often exhibiting two best frequencies.
These data support the hypothesis that glycine, GABA and aspartate effects in both DCN and VCN could frequently be reversed. Glycine required increased amounts of these substances to achieve amounts (0-50 nA) of GABA and glycine. However, few neurons which may govern transmission in the cochlear nuclei (CN) (Godfrey, 1977) displayed a pauser or build-up type pattern. Resulting 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 each putative neurotransmitter or antagonist being tested. Histological reconstruction of electrode tracks was carried out on all preparations, and patterns of PSTH's and ISIH's were used to aid in the localization of the recording sites. As previously reported (Caspy and Havey, Neurosci. Abs. 5, 1977), the application of glycine onto dorsal cochlear nucleus (DCN) neurons displaying a pause 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 sites from different ban of the same animal. Results in DCN neurons indicate that glycine inhibition occurred at lower doses than those for GABA. In ventral cochlear nucleus (VCN), glycine was found to have reduced the level of spontaneous activity to a greater extent than that of driven activity. Thus, complete inhibition of spontaneous activity resulting from the application of each neurotransmitter was achieved 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 with moderate to large amounts (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 antagonists. The relative effectiveness of the putative 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 NIH Research Fund)

A fundamental understanding of the nature of auditory thalamocortical pathways, for central as well as peripheral auditory systems, is important because of the role of auditory cortices in the processing of sound and because of the potential for treating disorders of auditory function. For central auditory systems, the study of other mammals is necessary because of the complexity of the mammalian auditory thalamus. In this study, retrograde transport of horseradish peroxidase (HRP) was used to study the organization of the auditory thalamus project to at least three subdivisions of the auditory cortex. The in vitro microphonic potential recorded in response to in vivo stimuli was analyzed. The delay of 40-50 µs. This short latency, and its modest sensitivity to temperature (Q10 = ~2.5), suggest that there is a distinct delay of 40-50 µs. This short latency, and its modest sensitivity to temperature (Q10 = ~2.5), suggests that there is a particular pattern of generalized thalamo-cortical linkages with unknown delays, and in part because analysis of the extracellular receptor potential (the microphonic) is complicated by complex current paths in the organ of Corti. We have investigated the response latency of hair cells with an in vitro system which largely circumvents these difficulties. The delay with which a sensory receptor responds to a stimulus provides information about the axes involved in transduction of the rapidly alter as neuronal signal. The latency of a response of hair cells, the primary receptor cells of the vertebrate acoustico-lateralis system, is not precisely known, in part because cells stimulated with low-level sounds have well maintained linkages with unknown delays, and in part because analysis of the extracellular receptor potential (the microphonic) is complicated by complex current paths in the organ of Corti. We have investigated the response latency of hair cells with an in vitro system which largely circumvents these difficulties. The latency of a response to a stimulus is determined by the time required for the stimulus to reach the sensory cell. We have used in vitro microphonic potentials recorded in response to a stimulus to determine the axonal time constant across the epithelium. The in vitro microphonic potential recorded in response to a stimulus 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 probe contact, and then measuring the potential across the outer hair cell membrane, we could measure within 5 µs and 0.01 µs 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. For correct characterization of the epithelial response waveform, the transfer function: V0 = V1 + (V1/V0)dt, the response waveform closely mimics that of the stimulus, with slight broadening and amplitude attenuation. When the transfer function is measured at the highest sensitivity to temperature (Q10 = ~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 effect of the interneuronal messengers diffusing over distances of greater than about 0.2 µm.
13 AUDITORY CORTEX LESIONS AND BRIEF TONE AUDIMETRY IN CATS: EVIDENCE FOR DISASSOCIATION BETWEEN DETECTION AND DISCRIMINATION ABILITIES. Jerry Cranford, Dept. of Otolaryngology, 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 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.

Distinct from 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 differences in discriminating 16 m sec. 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. This appears to be a situation of 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.

REFERENCES:

Cranford, J. L., & Igarashi, M. Brain Res. 136 (1977) 559-564

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, II. 60626.

The olotoliths 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, J. C. P. P. 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-noticeable difference for stimulus period, \( \Delta P \)).

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 microseconds at 1000 Hz. The behavioral \( \Delta P \) 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 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 determined by the phase-locking variance, then the threshold-like behavior to occur when the difference between the means (\( \Delta P \)) is approximately equal to the distributions' sd; that is, when \( d^* = \Delta P / \sigma_d \). 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.


We have investigated the descending projections from the auditory cortex of the galago, by employing 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 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 feature. 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 mammalian 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 to the magnocellular division of the medial geniculate body (GM). 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 thalamus and midbrain. Regarding the descending pathways from cortex to the midbrain, a distinctive difference between the projections of Ak and the surrounding belt regions was observed. The koniocortex projections target the central nucleus of the inferior colliculus and the terminations are distributed in a laminar fashion. On the other hand, the descending projections terminate in the pericentral nucleus of the inferior colliculus and in the deep layers of the superior colliculus. All of these corticointercollicular pathways originate in koniocortex.

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 affenter input. (Supported by NIMH grant MH-4649 and NIMH fellowship MH05964.)


We have investigated the recovery of spike discharge probability of cat cochlear nerve fibers as a function of interspike time by collecting histograms of the intervals between spikes with and without single tone stimulation. Estimates of \( \phi(t) \), the hazard function (Cox, Renewal Theory, J. Wiley, 1962), were formed by dividing the number of intervals of length \( t \) by the number of intervals of length greater than or equal to \( t \). In most fibers, \( \phi(t) \) for spontaneous discharges increased rapidly between \( t \) of 1 and 4 msec, and continues to increase more slowly between 4 to 40 msec. Observed differences among fibers include a tendency of some fibers to show an "early peak" in \( \phi(t) \) 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, \( \phi(t) \) was nearly equal to \( \phi(t) \) 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 pattern in response to tonal stimulation (>5 kHz) tones. These predictions will be compared with experimental results.
Amplitudes of auditory evoked potentials change readily with repetition rate and duration of acoustic stimulation. A quantitative model was proposed to explain how such changes are due to refractory mechanisms at the hair-cell and acoustic nerve junction. The decrement was smaller relative to the results from spontaneous recovery shortened and the amount of response decrement is determined by the time constants for spontaneous recovery processes, respectively. Interactions of these mechanisms during acoustic stimulation the time constants were measured. 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 negativity periods of auditory periphery and central elements.

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 an organ of Corti. The NM nerve was recorded on the 8th nerve stumps. Using microelectrodes filled with potassium citrate or horseradish peroxidase (HRP) solution, we have recorded field potentials 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 negativity periods of auditory periphery and central elements. 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 evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 8-10ms longer than that of N1; this is consonant with the view that the 8th nerve does not project directly to NL, and that N2 evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 10-15 and 100-120 msec for the decrementing and recovery periods of auditory periphery and central elements, 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. Electrical stimulation of the paralyzed nerve stimulation are characterized by three distinct negativity periods of auditory periphery and central elements. Interactions of these mechanisms during acoustic stimulation the time constants were measured. The findings summarized below were obtained from late embryos, aged 17-19 days.

Electrical stimulation of the 8th nerve does not project directly to NL, and that N2 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 evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 8-10ms longer than that of N1; this is consonant with the view that the 8th nerve does not project directly to NL, and that N2 evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 10-15 and 100-120 msec for the decrementing and recovery periods of auditory periphery and central elements, 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. Electrical stimulation of the paralyzed nerve stimulation are characterized by three distinct negativity periods of auditory periphery and central elements. Interactions of these mechanisms during acoustic stimulation the time constants were measured. The findings summarized below were obtained from late embryos, aged 17-19 days.

Electrical stimulation of the 8th nerve does not project directly to NL, and that N2 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 evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 8-10ms longer than that of N1; this is consonant with the view that the 8th nerve does not project directly to NL, and that N2 evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 10-15 and 100-120 msec for the decrementing and recovery periods of auditory periphery and central elements, 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. Electrical stimulation of the paralyzed nerve stimulation are characterized by three distinct negativity periods of auditory periphery and central elements. Interactions of these mechanisms during acoustic stimulation the time constants were measured. The findings summarized below were obtained from late embryos, aged 17-19 days.

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

The pinnae of Holothane anesthetized cats were removed and an ear piece was placed in the external auditory meatus. A Beyer DT-48 earphone was coupled to each ear piece by a short plastic tube. Pt-1r electrodes were advanced into primary auditory cortex using a hydraulic system from the outside of the skull. The electrode was connected to a double-wall acoustic chamber. Fifty msec best frequency tone bursts (rise/fall time 5 msec) presented at moderate stimulus levels relative to the unit's threshold were used. Stimulus level was equal at the two ears.

Inter-aural time differences between the contralateral stimulus leading by 50 msec and the ipsilateral stimulus leading by 50 msec were explored. The most common effect observed was a reduced discharge rate when stimulation of the ipsilateral ear preceded 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. The duration of the pattern 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 delay functions to the lateralization of a sound source could derive from intensity differences at the two tympanic windows due to the presence of the head in the sound path to the distant. This difference approaches 10 db for high frequencies. At near threshold levels mean response latency of AVCN cells decreases by several msec with each 10 db intensity increase (Kittes et al. J. Neurophysiol., in press). The largest intensity 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.


The spatial receptive fields of specialized auditory units in the midbrain of the barn owl (Tyto alba) are subdivided into separate excitatory and inhibitory responses. Separate single cell response fields similar to the contralateral 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 (mesencephalic nucleus 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 respective 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. All the source placed 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, particularly at sources near the unit's auditory area toward the periphery. Tone bursts also inhibited these units, but the effect was weaker. The range of sound frequencies to which the auditory unit was sensitive varied with the 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 has important implications for the understanding of auditory processing in the brain. The center-surround organization could provide a way to code the location of a sound source. This is consistent with the idea that the auditory system has created center-surround receptive fields to code the location of a sound source. This is consistent with the idea that the auditory system has created center-surround receptive fields to code the location of a sound source.

26

Sporontaneous and sound-activated medial geniculate (MG) single unit discharges were studied in chronically implanted, unanesthetized, paralyzed cats. Comparisons were made between activity during high-voltage ("h") and low-voltage ("l") EEG periods (i.e. different modes of activity) 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. Numerous stimulus repetitions 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 division cells.

Our spontaneous discharge rate data suggest that descending fibers from the auditory cortex are predominantly excitatory and of the subtype modulatory variety. Since the effect on MG neuronal activity of "h" (corticofugal) was much the same as the effect of "c" (reduced corticogeniculate discharge), one consequence of reduced arousal seemed to be a reduction in corticofugal modulating discharge to the MG. Increased burst activity and reverberations during sleep and cortical cooling may be "re-leased" among relay and local circuit elements as a result of decreased cortical input. These may be less extreme examples of the same phenomenon that produce bursting and enhanced reverberation in the presence of barbiturate anesthesia.
THE EFFECT OF SIMULATED SONIC BOOMS ON THE INNER EAR STRUCTURE AND FUNCTION. Stanislav Ereits, Chris Tasros* 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.

SOCIETY FOR NEUROSCIENCE

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 eleven 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 interspike intervals between the first wave and successive waves. Several changes in the form of the waves appeared across development. As it emerged, wave I had two distinct components which tended to merge as the animal 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 young 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.)


Previously we reported that injection of a single 400 mg/kg dose of kanamycin (KAN) s.c. followed by a single 10 mg/kg dose of ethacrynic acid (EA) i.v. resulted in the rapid destruction of outer hair cells (OHC) (Fed. Proc. 36: A12, 1977). Early OHC damage, visible within 2-1/hr 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 ENA/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/FAL. 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 SV 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 damage to the 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 MRCIC 5901 NS 15080-03.)


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 cochleas 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 a substance may be considered as a temporary threshold shift. (Supported by NS # 11647, The Veterans Administration Research Service and The John A. Hartford Foundation).

The inferior colliculus of bush baby can be divided, based on its cytoarchitecture, into a central nucleus which is surrounded dorsally, medially, and laterally by striate nuclei and is surrounded laterally and rostrally by an external nucleus. The central nucleus can be further subdivided into dorsomedial and ventrolateral diagonal sectors. To discover the different projections to these subdivisions, horseradish peroxidase was electrophoretically injected into the inferior colliculus of 20 bush babies at 1 week of age and the tissue processed with diaminobenzidine (DAB), catechol/p-phenylenediamine, hematoxylin dihydrochloride (HDC), or thionin-ethylenediamine tetracetic acid (TB). Labelled and unlabelled axons and terminals were found in the central auditory nucleus and then converted to a percentage of the total number of labelled cells found in each case.

Considering the inferior colliculus of bush baby, the results indicate that it receives bilateral input from the dorsal and ventral cochlear nuclei, lateral superior olives and the dorsal nucleus of the medial geniculate body (MSO). However, the ipsilateral projection from the cochlear nucleus is not nearly as strong as the contralateral projection, while the opposite inferior colliculus is revealed only by the more sensitive processing techniques, it probably represents collateral connections from the ipsilateral lateral pathway. Exclusively unilateral pathways arrive at the inferior colliculus, one originating in the ipsilateral ventral cochlear nucleus, lateral superior olives and the dorsal nucleus of the medial geniculate body (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 lateral pathway. Exclusively unilateral pathways arrive at the inferior colliculus, one originating in the contralateral ventral cochlear nucleus, lateral superior olives and the dorsal nucleus of the medial geniculate body (MSO), but not to the ventrolateral diagonal sector. These mutually exclusive projection areas suggest that interaction between third order auditory nuclei, which are primarily sensory in character, and sixth order auditory nuclei (which are probably motor in nature) is accomplished via complex interneuronal connections within the inferior colliculus itself.

(Published by NIH postdoctoral fellowship NS-05564.)

DUAL OLIVOCOCHLEAR BUNDLES: DIFFERENTIAL ORIGINS AND TERMINATIONS IN THE CAT. W. Bruce Warr and John J. Guinan, Jr.* The Boy's Town Institute for Communication Disorders in Children, Omaha, Nebr., 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 radioactively labelled proteins, and teratocarcinoma (TDI) and teratocarcinoma (TDII) and teratocarcinoma (TDIII). The left ears of six other animals show the combined effects of the two surgeries; where portions of the organ of Corti were removed or degraded, the second surgery removed or destroyed the hair cells in the affected area. The left 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 remained; the ears show a degerated nerve. The left ears of six other animals show the combined effects of the two surgeries; where portions of the organ of Corti were removed or degraded, the second surgery removed or destroyed the hair cells in the affected area. The left ears were serially sectioned and stained with Luxol fast blue-cresyl violet to demonstrate neurons and myelinated fibers.


The cochlear nucleus comprises distinct cell groups, whose synaptic connections form the basis for all further integration in the auditory system. The posteroventral cochlear nucleus of the cat, as visualized with the Nissl stain, contains two types of neurons. Globular neurons are characterized by distinct and plump synaptic vesicles, and pleiomorphic synaptic vesicles, respectively. Terminals of the spiral ganglion neurons which make contact with hair cells do not synapse on dendritic terminals of hair cells, suggesting that spiral ganglion neurons play a significant role in determining the properties of the projection to the inferior colliculus.

(Published by PHS grants NS 06115, NS 13126, GM 00404, NH 14275, and NS 14347.)
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 p•
raffin sections stained with thionin or by the Protargol technique to ob-
tain information about the organization for both histological and electrophysiological studies. These data were correlated with radioautographs showing the distribution of primary auditory terminais labeled by perilymphatic per-
fusion with 3H-proline. The ventral cochlear nucleus (VCN) has a homogenous population of spherical neurons. Dorsally these neurons are larger and more rounded than in the remainder of the region. The smallest and most densely packed spherical cells are found in the rostrolateral area (RL). In autoradiographs, spherical and neurons are encircled by dense accumulations of silver grains which presumably are labeled end bulb of Held. Caudally, the spherical neurons are replaced dorsally by small and medium-sized neurons. These are round or elliptical and are interspersed with scattered giant cells. The rostral extent of these neurons is more medially than laterally. Ventrolaterally, the rostral AVCN is bounded by a globular region which is often aligned along the ascending branch of the auditory nerve. In radioautographs, the g•
lobular 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 por-
tion of the posterior ventral 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 number of neurons which include small, medium, and large round 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 neuron types in PVCN. In the laminar structure of the dorsal cochlear nucleus (DCN), pyramidal cells are ar-
nanged 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 are rounded, are found throughout the DCN. The density of silver grains on 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 R/L. (Supported by NIH grant NS 38590 to RLG.)


The neuronal organization of the inferior colliculus (IC) of the albino mouse was examined in Golgi, Nissl and fiber-stained materials (two methods). Two major subdivisions: (1) an 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 inferior colliculus, and (2) a cell body area, or cortex, 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, the CN resembles the dorsal nucleus of the albino mouse, with larger superficial neurons which project to the midbrain tegmentum and outputs to the auditory cortex. Superimposed on this are smaller, more compact, multipolar neurons which project to the midbrain tegmentum and outputs to the auditory cortex. In the anterior part of CN, many of these neurons are surrounded by punctate accumulations of silver grains which presumably are labeled end bulbs of Held. Caudally, the CN has many globular cells, which are often aligned along the ascending branch of the auditory nerve. In radioautographs, the g•
lobular 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 por-
tion of the posterior ventral 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 number of neurons which include small, medium, and large round 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 neuron types in PVCN. In the laminar structure of the dorsal cochlear nucleus (DCN), pyramidal cells are ar-
nanged 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 are rounded, are found throughout the DCN. The density of silver grains on 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 R/L. (Supported by NIH grant NS 38590 to RLG.)
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/, /E/, /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 dB SPL) 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.

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, 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 Β-AMINOBUTYRIC ACID (GABA) ANTAGONISTS. Susan A. Berman, Craig A. Johnson* and Gerald L. Geber. Dept. of Pharmacol., Michigan State Univ., East Lansing, MI 48824. Geber and Snyder (Am. J. Physiol. 218: 134, 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 basal heart rate in high spinal cats. Experiments were performed on unanesthetized, high spinal (C1 transected) cats which were artificially resired 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, H0 to H4) 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 effect 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 HL11387.)

44 A SPINAL SYMPATHO-INHIBITORY ACTION OF CHLORPROMAZINE IN THE CAT. Patricia J. Bernthal* and Michael C. Koss. Dept. Pharmacology, Univ. Okla. Health Sciences Cent., Oklahoma City, Oklahoma 73190 Chlorpromazine (CPZ) has been shown to depress several autonomic systems (Schallek & Zabransky, Arch. Int. Pharm. Ther., 1966, 181, 128; Sieg 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 (Davisson & Koss, Neuropharmacology, 1976, 15, 197) or reflex (Bernthal & Koss, Neurosci. Abstr., 1977, 3, 17) stimulation. An observed lack of effect of CPZ on the peripherally evoked EDR (Davisson & 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 C1. 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 C3 with a submaximal frequency. CPZ depressed the electrodermal response in a dose-dependent fashion. The mean ED50 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 ADRENERGIC PATHWAYS IN THE CONTROL OF ARTERIAL PRESSURE IN NORMOTENSIVE RATS. Luiz A.A. Camargo*, Wilson A. Saad, Jose A.C. Machado*, Gildo W.A. Augusto and Vandy J. Meiners. Inst. of Neuroi., SPPR, Dent., UNESP, Araraquara, SP, Brazil, 14,800.

In studies of central regulation of arterial pressure related to cardiovascular effects, several areas were studied with adrenergic and adrenolytic substances, and dual responses, with 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. Rates with carumae implanted into the hypothalamus were injected with 20 nmol noradrenaline, alone, or preceded by administration of phenolamine and propyramol 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, SD ± 7 from a baseline of 90 mmHg) and blocked by the β- adrenolytic agent propranolol, but not by the α- adrenergic agent phenolamine, the other receptor was hypotensive (Mean of 15 rats= 31 mmHg, SD ± 5 from a baseline of 98 mmHg), and blocked by phenolamine. These results lead us to postulate the existence 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, SD ± 6 from a baseline of 102 mmHg), and hypotension by β receptors (Mean of 20 rats= 41 mmHg, SD ± 8 from a baseline of 55 mmHg). Supported by Grant FAPERJ.


In studies of central regulation of arterial pressure related to cardiovascular effects, several areas were studied with adrenergic and adrenolytic substances, and dual responses, with 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. Rates with carumae implanted into the hypothalamus were injected with 20 nmol noradrenaline, alone, or preceded by administration of phenolamine and propyramol 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, SD ± 7 from a baseline of 90 mmHg) and blocked by the β- adrenolytic agent propranolol, but not by the α- adrenergic agent phenolamine, the other receptor was hypotensive (Mean of 15 rats= 31 mmHg, SD ± 5 from a baseline of 98 mmHg), and blocked by phenolamine. These results lead us to postulate the existence 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, SD ± 6 from a baseline of 102 mmHg), and hypotension by β receptors (Mean of 20 rats= 41 mmHg, SD ± 8 from a baseline of 55 mmHg). Supported by Grant FAPERJ.

Clonidine-induced hypotension and bradycardia have been attributed to its activation of postganglionic adrenoceptors in the medulla, resulting in a modification of the autonomic cardiovascular outflows. Based on microinjection and lesion experiments, Chen and Chan (1977) have identified in the rat that the medial medullary reticular formation (MMRF) may be a critical bulbar site that is related to such clonidine-elicited cardiovascular effects. Further investigations of the roles played by the α-adrenoceptors in the MMRF and vagus nerve in the clonidine-elicited cardiovascular effects.

Intravenous injection of clonidine (10 µg/kg) produced an initial, transient rise in arterial blood pressure, followed by a prolonged hypotension lasting 35 min. Tail bradycardia was also observed for 60 min postinjection. In animals pretreated with haloperidol, an α-adrenoceptor blocking agent, which was injected 10 min after clonidine (10 µg/kg), no hypotensive or bradycardic responses were observed. In contrast, clonidine injection of 10 µg/kg could only elicit the initial vasoconstrictor response (vasopressor effect) without hypotensive or bradycardic responses. In cats receiving unilateral vagotomy, only the initial vasopressor response was observed. However, unilateral vagotomy in these spinal animals. On the other hand, the responses could be abolished by either the administration of atropine or bilateral vagotomy in these spinal animals. The results suggest that the clonidine-induced hypotension and bradycardia in cats may be mediated by the α-adrenoceptors in the MMRF, 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.)


Chronic indwelling tail arterial cannulae (Chiueh and Kopin, J. Pharmacol. Exp. Ther. 205: 148, 1976) were used for monitoring of blood pressure and withdrawal of blood samples. Arterial blood samples (0.5 ml) were taken before and after administration of uncarine A (50 mg/kg, s.c.), clonidine (0.49 mg/kg, i.v.) 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 activity of the sympathetic nervous system.

Intravenous injection of clonidine (10 µg/kg) produced an initial, transient rise in arterial blood pressure, followed by a prolonged hypotension lasting 35 min. Tail bradycardia was also observed for 60 min postinjection. In animals pretreated with haloperidol, an α-adrenoceptor blocking agent, which was injected 10 min after clonidine (10 µg/kg), no hypotensive or bradycardic responses were observed. In contrast, clonidine injection of 10 µg/kg could only elicit the initial vasoconstrictor response (vasopressor effect) without hypotensive or bradycardic responses. In cats receiving unilateral vagotomy, only the initial vasopressor response was observed. However, unilateral vagotomy in these spinal animals. On the other hand, the responses could be abolished by either the administration of atropine or bilateral vagotomy in these spinal animals. The results suggest that the clonidine-induced hypotension and bradycardia in cats may be mediated by the α-adrenoceptors in the MMRF, which in turn facilitate the vagal outflow to the heart, resulting in clonidine-induced hypotension and bradycardia.


Sustained arterial pressure 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. Previous studies have reported that the area postrema (AP) lies in close apposition to the solitary tract nucleus (NTS) immediately ventrolateral to it. 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. We were interested in whether the AP, most afferent connections were obtained on 8 adult mongrel dogs according to the methods of Ramon-Moliner (Stain Technol. 32: 19, 1950; Stain Technol. 39: 65, 1964). Sections were cut serially at 50-150 µm in the transverse plane. Orientation purposes alternate sections were counterstained with cresyl violet. The Bodian silver technique (Bodian, Anat. Rec. 83: 349, 1943), used to stain the cresyl violet, was done on 10 µm 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, containing capillaries and a highly vascular surface. In the same area some of the neuronal cell bodies are located at the ventricular surface, while others have a slight to no ventricular incorporation. The region is characterized by the complexity of the adventitia and the surface. Complex connective tissue adjacent to the adventitia appear to be unique to the canine AP. The mantle area has long-lasting hypotensive effect in SHR rats but not in normotensive rats. The results suggest that the canine AP has a direct projection from the nucleus tractus solitarius (NTS) and receives a direct projection from the paraventricular nucleus (PVN). In addition, the AP receives a direct projection from the paraventricular nucleus (PVN) which is abundant in the junctional zone where there are delicate processes extending dorsally into the intermediate medullary layer and ventrally to the NTS. These observations raise questions concerning the role of the dog's area postrema in cardiovascular control. (Supported by MRC of Canada)
A study was undertaken to determine the effect of biofeedback induced changes in skin temperature 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 rebound elevation 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, T, hand-cooling 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 3He inhalation technique. One rCBF run was given during a relaxation condition in which the subject was 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 intracranial or common migraine and having a minimum of two migraine attacks per month. Each volunteer was randomly assigned to either a hand-warming, T, hand-cooling or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

Comparisons 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, T, hand-cooling or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

The prodrome stage involves the reduction of intracranial blood flow followed by a rebound elevation 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, T, hand-cooling 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 3He inhalation technique. One rCBF run was given during a relaxation condition in which the subject was 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 intracranial or common migraine and having a minimum of two migraine attacks per month. Each volunteer was randomly assigned to either a hand-warming, T, hand-cooling or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

Comparisons 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, T, hand-cooling or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

The prodrome stage involves the reduction of intracranial blood flow followed by a rebound elevation 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, T, hand-cooling 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 3He inhalation technique. One rCBF run was given during a relaxation condition in which the subject was 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 intracranial or common migraine and having a minimum of two migraine attacks per month. Each volunteer was randomly assigned to either a hand-warming, T, hand-cooling or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

Comparisons 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, T, hand-cooling or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

The prodrome stage involves the reduction of intracranial blood flow followed by a rebound elevation 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.
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-CX). 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 (DNX) and an intermediate zone (IZ) between the NA and DNX. In each animal about 78% of the soma were in the NA while 17% and 5% were located in the DNX and IZ, respectively. Approximately 72% were fusiform and 28% were round in shape. The average soma length was 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 identified with non-cardiac tissue or the result of HRP uptake by ganglion cells in the IMG of the cat. Two types of cells were impaled: inexcitable and excitable cells. Ten percent of the cells impaled were inexcitable and had a high resting membrane potential (up to 75 mV). They had a low excitability and showed no response to a direct intracellular depolarizing current. Their membrane potential ranged from -45 to -85 mV (mean -70 mV). They were insensitive to stimulation of hypogastric, intermesenteric, inferior mesenteric (IMG) nerves. Excitatory potentials were recorded in 40% of the cells and were evoked by stimulation of the sympathetic system.

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 sec 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 NIH grant HL08682).
METABOLIC MAPPING OF NEURAL PATHWAYS INVOLVED IN GASTROSECRETORY
stimuli.

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 150 mg/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 [14C]-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 [14C]-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 the eighteen structures examined, including, for example, the ventromedial medial nucleus of the hypothalamus, globus pallidus, zona incerta and n. ambiguus, the glucose consumption unchanged and 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, perifornical area, dorsal motor nuclei 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 were also to be involved in the gastro-secretory response to the blockade of glucose utilization provoked by pharmacological doses of 2-deoxyglucose (Kadakor et al., Neuroscience 29:291, 1977) suggesting that these same nuclei are also activated by hypoglycemia.


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 sympathetic activity during these two stimuli. Fifteen cats were anesthetized with chloroform and chloralose-urethane (40-80 mg/kg). A proximal jejunal loop was made and the vasculature of the loop was dissected. The blood flow of the jejunal loop was measured by using a side arm perfusion method. The jejunal loops were divided 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 unaccompanied with cardiac rhythms, became synchronized with phrenic activity during slight hyperventilation or noventilation. 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 26% of vertebral nerve fibers, showed irregular firing patterns which could not be made synchronous with phrenic nor cardiac rhythms by various maneuvers. They were affected by activation of baroreceptors but increased their activity greatly under asphyxia. It is hoped that such analyses of single postganglionic fibers will give more information concerning the properties of postganglionic sympathetic fibers in gastro-intestinal and vascular smooth muscles. Moreover, these populations may be activated independently by different stimuli.

PATTERNS OF SINGLE UNIT ACTIVITY IN SYMPATHETIC POSTGANGLIONIC NERVES (CARDIAC AND VERTEBRAL NERVES). Mark Kollai and Kiyomi Kotani. Dept. of Physiol., 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. The fibers were divided 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 unaccompanied with cardiac rhythms, became synchronized with phrenic activity during slight hyperventilation or noventilation. 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 26% of vertebral nerve fibers, showed irregular firing patterns which could not be made synchronous with phrenic nor cardiac rhythms by various maneuvers. They were affected by activation of baroreceptors but increased their activity greatly under asphyxia. It is hoped that such analyses of single postganglionic fibers will give more information concerning the properties of postganglionic sympathetic fibers in gastro-intestinal and vascular smooth muscles. Moreover, these populations may be activated independently by different stimuli.

BARORECEPTORS, CATECHOLAMINES, NUCLEUS SOLITARIUS, AND THE AREA POSTEROM: A CARDIOVASCULAR CONNECTION. David W. Rats and Harvey J. Harten. Dept. of Biology (ICM) and Psychiatry (HJK), 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 (DM) to the nucleus solitarius (nS) in pigeons. The distribution of DM fibers was compared with the projections of nS noradrenergic neurons within nS as revealed by fluorescence histochemistry. The relationship between the DM 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 3-5 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 sucalis 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 tenua chordae.

The region surrounding the tenua chordae had previously been suggested to encompass the area postrema in birds (Moll, J. and Hilleberg, C. Proc. Konin. Ned., 24, 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 intraventricular injections of HRP (Broadwell, R.O., Brightman, M.W., J. Comp. Neur., 166, 1976). This technique revealed that the rostral portion of the area postrema lies immediately adjacent to the caudal portion of Sd. The distribution of HRP within the neuropil of Sd overlapped precisely with the DM projection area. Fluorescent fibers extended from the caudal portion of Sd into the adjacent area postrema and tenua chordae. These data indicate that depressor afferents have a restricted distribution within the nucleus solitarius in pigeons. Our data also provide strong evidence for the involvement of catecholaminergic neurons in the cardiovascular sensory pathway. In addition, our data suggest an anatomical association between the area postrema, which lacks a blood-brain barrier, and the respiratory projections of the area postrema. Connections between these two regions may be partly responsible for the cardiovascular effects of blood-borne substances, such as angiotensin II, which are carried to the central nervous system.

Positive pressure lung inflation in open chested dogs resulted in a marked latency inhibition of cardiopulmonary sympathetic efferent neural activity (CPSENA) before and after bilateral vagotomy. Mongrel dogs, 20-30 kg, were anesthetized using sodium pentobarbital, 35mg/kg i.v. and placed on a positive pressure ventilation. The chest was split transversely between the second and third ribs, to allow unimpeded access to both stellate ganglia and adjacent anastomosis. Inhibina was recorded from one central end of the right or left 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 (ECC) was monitored from leads placed in a lead II configuration. Averaged CPSENA, ECC and the pressures were recorded using a polygraph.

Prior to vagotomy, lung inflation to 15mm Hg resulted in a marked inhibition of CPSENA traversing the left and right ansae subclaviae. At the onset of expiration, CPSENA immediately returned or momentarily exceeded pre-inflation levels. Each inflation consistently 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 tetanus. This effect may have been due to the marked depression of baseline CPSENA produced by vagotomy. Bilateral sympathectomy by sectioning the remaining intact ansae resulted in a further depression of baseline CPSENA. Lung inflation to 15mm Hg in these animals 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 breathe spontaneously. During expansion of the chest, CPSENA was immediately depressed without lung inflation. This study suggests that either chest wall somatic afferents or centers in the brainstem may influence CPSENA. This study has also provided some evidence indicating that some vagal and sympathetic cardiopulmonary afferents can have an excitatory effect on CPSENA. This effect may be modified by vagal and sympathetic cardiopulmonary afferents that can have an inhibitory effect on CPSENA. (Supported by Grant HL 16511 and Young Investigator Research Award HL 21042 from NHLBI, and the Medical Research Service of Wisconsin and Wood VA Ctr., Milwaukee, WI 53193)


Electrophysiological experiments on the abdominal prevertebral ganglia of the pig indicate that there is a vasoregulatory 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 single ganglia, 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 caudal IMG. Solid horseradish peroxidase (HRP) was placed on crushed regions of these ganglia. Labelling of sympathetic nerve trunks 5 to 8 mm from the ganglia. After 1-6 hours the preparation was fixed and 42 µm thick frozen sections of all the ganglia were processed in benzene. Immunoperoxidase was placed on the lumbar colonic nerves, labelled neurons were identified 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. Moreover, the lumbar pattern of innervation was 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 possibly some of the afferents to the celiac ganglia originate from the cephalad CG and the caudal SMG network and that there is some crossover of this outflow. That is, some neurons located in the cephalad ganglia pass through the caudal ganglia and out caudal nerves. Also, some neurons from the caudal 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.)
67 IDENTIFICATION OF VISCERAL AFFERENTS TO THE SACRAL CORD OF THE CAT USING HORSESEADISH PEROXIDASE. Charles Morgan, William C. de Groat, Irving Heidelberger, Dept. Pharmacol., Univ. of Pittsburgh Sch. of Med., and V.A. Hospital, Pittsburgh, PA 15261

The parasympathetic preganglionic afferents to the pelvic viscera leave the sacral spinal cord and travel toward the pelvic nerve before innervating 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 and exposing the central portion of the spinal cord (SC) at the mid-S2 level and allowing 35-60 hrs. transport time, we have been able to label both the afferents and efferents from the pelvic viscera to the sacral cord. Frozen sections of spinal cord (50 µm) 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 was identical to that observed in the other cats. The distribution of HRP and allowing 35-60 hrs. transport time, we have been able to label both the afferents and efferents from the pelvic viscera to the sacral cord. Frozen sections of spinal cord (50 µm) 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 was identical to that observed in the other cats.

68 BAROREFLEX REFLEX GAIN DURING HYPOTHALAMIC ACTIVATION IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS. S. Morrison and D. Whitburn, Dep. of Physiology and Biophysics, Univ. of Vermont, Burlington, VT 05405.

Baroreflex mechanisms in the cat, including those involving the baroreceptors, are complex and involve multiple afferent and efferent pathways. The baroreceptors are located in the aortic arch and the carotid sinus, and their activity is modulated by central nervous system (CNS) mechanisms. Baroreceptor reflexes are important in the regulation of blood pressure, heart rate, and other cardiovascular parameters. The reflex gain is a measure of the sensitivity of the baroreceptor reflex and is an important factor in the development of hypertension. The baroreflex gain is measured using a variety of techniques, including recordings of blood pressure, heart rate, and other cardiovascular parameters in response to changes in blood pressure or other stimuli.

The baroreflex gain is measured using a variety of techniques, including recordings of blood pressure, heart rate, and other cardiovascular parameters in response to changes in blood pressure or other stimuli. The baroreflex gain is an important factor in the development of hypertension, and an increased gain is associated with an increased risk of hypertension. The baroreflex gain is measured using a variety of techniques, including recordings of blood pressure, heart rate, and other cardiovascular parameters in response to changes in blood pressure or other stimuli.

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 neuronal membrane transport system. Studies of the MRF (50 pulses, 0.5 msec, 200 cps) during expiration (E) caused a phasic-switch to inspiration (I). In some cases 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 6.3 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 and branches to the gut wall have been processed in the same high pressure sides of the circulatory system. Studies of the MRF (50 pulses, 0.5 msec, 200 cps) during expiration (E) caused a phasic-switch to inspiration (I). In some cases 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 6.3 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 and branches to the gut wall have been processed in the same high pressure sides of the circulatory system.

The factor was fairly constant at all intensity-delay combinations. For E-to-I, the average factor across Ss, was the delay in seconds after which the stimulus stimulation provided complete control of respiration. The factor was fairly constant at all intensity-delay combinations. For E-to-I, the average factor across Ss, was the delay in seconds after which the stimulus stimulation provided complete control of respiration. The factor was fairly constant at all intensity-delay combinations.

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 pentobarbital (35 mg/kg, i.v.) and placed on positive pressure ventilation. The second to fifth ribs along with the intercostal arteries 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 and retained for stimulation. The left sympathetic chain and T2-T4 white rami communicantes were isolated by sectioning the chain below T4 and each white ramus at its junction with the spinal nerve. Individual bundles were sequentially placed on tungsten-carbide recording electrodes which were connected to high gain preamplifier 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 1 mA, 0.5 msec, and 12-15 ma resulted in the stimulation of both Aδ and C fibers. Stimulation of each nerve, while recording from T2-T4 white rami, evoked a greater percentage of C fibers as compared to Aδ fibers. The percentage of cardiac afferent fibers (2,1-16.4/sec). Stimulation of the SCN and VMCN produced a greater number of potentials than did stimulation of the VLCN. The VLCN contained fewer fibers with conduction velocities greater than 2 m/sec. There did not appear to be any preference distribution of cardiac afferent fibers arising from any ramus examined in this study (T2-T4). 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.)


Silver techniques have previously been used to demonstrate the visceral motor and parasympathetic fibers that originate from the dorsal motor nucleus of the vagus. There is minimum of information concerning the anatomical relationships of these preganglionic fibers within the bronchial and epicardial 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 epicardial 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 105 microcuries of tritiated leucine. Following a postoperative survival period of three days, the animals were perfused with 250 micro-curies of tritiated leucine. A selective postsynaptic blockade by gallamine or pancuronium suggested from the surface potential studies was evaluated further by the histochemical fluoride technique of Falck et al. (J. Histochcm. Cytochcm. 10:348-354, 1962). In vitro exposure of rat superior cervical ganglion (SCG) to betahanechol, a muscarinic stimulant, reduced fluorescence in both ganglionic neurons and interneuronal (SI) cells. Treatment of rat SCG with betahanechol (in vivo) and concomitant with betahanechol (in vitro) or pancuronium (in vivo) prevented the decrease in SI cell fluorescence resulting from in vitro exposure to betahanechol. Pretreatment of the SCG with pancuronium alone had no observable effect on either ganglionic neuronal or SI cell fluorescence. A bundle of the SCG was placed in 35 mg/kg of the SCG in vivo as well as during in vitro exposure with betahanechol produced a depletion of SI cell and ganglionic neuronal fluorescence comparable to that from betahanechol 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 of two pharmacologically distinct muscarinic receptors (M1 and M2) in sympathetic ganglia.


Electrical recordings of the superior cervical ganglionic surface potential in cats indicated that gallamine or pancuronium inhibit the muscarinic mediated hyperpolarization of the ganglionic cell. The selective postsynaptic blockade by gallamine or pancuronium without affecting slow depolarization (S-EPSP) by selective haloperidol to protect against histofluorescence depletion (NFEPSP) by selective 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 of two pharmacologically distinct muscarinic receptors (M1 and M2) in sympathetic ganglia.
81 ACUTE CHANGES IN BODY FLUIDS AFTER BARORECEPTOR DENERVATION.


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 (MAPB). Guyton postulated that any rise in BP due to interruption of baro-receptor 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 Macklem: Fed. Proc. 37:763, 1978). Activation of cardiac sympathetic afferent fibers by intravenous or topical application of bradykinin, prostaglandin E2, or histamine increased renal nerve activity by 17-131%. The duration of the response was 1-2 min. The increase in renal nerve activity was apparent upon repeated administration of one concentration of bradykinin. Blood pressure responses were attenuated and renal nerve responses eliminated by removal of the renal nerves in the left T2 DRG. Fluid volume and renal function were not determined in these latter studies. 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 E2 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 E2 or histamine can reflexly increase renal nerve activity. This reflex may be responsible for some of the changes in kidney function associated with cardiac dysfunc-

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 10th paravertebral ganglia were injected with 30% horse-radish 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 opened, the LADA was identified and 15 injections consisting of levels C7 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 #1-1951-488009.
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 accessible 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 post-ganglionic 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 ganglion 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
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. Hash. Dept. Psychol., Ft. 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 Künze, 1974) the lateral reticular nucleus (Künze and Cuénod, 1975) and the dorsal column nuclei (Berkley, 1975) of the cat. In these experiments, 3H-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 6H-proline is dissolved in a solution of calcium chloride and injected into the dorsal column nuclei of cats, the 3H-proline appears to be incorporated by large as well as by small neurons throughout the injection site. If the 6H-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 isoccasioned in part by some active property of the membrane of the large cells.

Supported by PHS grants KO4 NS 00118, ROI NS 11892 and NSF grant BSS 76-10335.

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.


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. In this study, 3H-NGF impregnated pellets of polycrylamide 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 3H-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 in part by NS50721 and Jerry Lewis Neuromuscular Disease Research Center grant.)

Proteins in the wavefront of fast axonal transport. Mark A. Blasy, Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

L-[35S]-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 35S 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). Photographs 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 (5 and 5') 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 was predicted by the filament hypothesis of Ochs (J. Physiol. 227, 627-645, 1972) and the rapid axonal transport of protein (Ochs: J. Physiol. 227, 627-645, 1972). 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.)

Retrograde transport of HRP to lumbar spinal ganglia. M. A. Blasi and T. J. Beall. Neurology, University of Massachusetts, Neurological Unit, Wright State University, Dayton, Ohio 45435.

HRP (Proshansky & Egger, Neurosci. Lett., 1977) may be used as a tracer to map sensory connections. Few sensory connections abolished in matching sections processed with HRP. 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 capillaries (10 μl) before they were placed in a perfused rat. 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-V1); (3) the nucleus gracilis; (4) the intermediolateral column and ventral horn; (5) the nucleus gracilis in the medulla. When compared with 24 h survival, 6 h 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 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.

(Retronadegate 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-V1); (3) the nucleus gracilis; (4) the intermediolateral column and ventral horn; (5) the nucleus gracilis in the medulla. When compared with 24 h survival, 6 h 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 so far been observed in the cerebellum (Felix and Künze, 1974) the lateral reticular nucleus (Künze and Cuénod, 1975) and the dorsal column nuclei (Berkley, 1975) of the cat. In these experiments, 3H-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 6H-proline is dissolved in a solution of calcium chloride and injected into the dorsal column nuclei of cats, the 3H-proline appears to be incorporated by large as well as by small neurons throughout the injection site. If the 6H-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 in part by some active property of the membrane of the large cells.

Supported by PHS grants KO4 NS 00118, ROI NS 11892 and NSF grant BSS 76-10335.
88 ANTEROGRADE TRANSPORT OF HORSERADISH PEROXIDASE IN THE VISUAL SYSTEM OF THE TREE SHREW. Russell G. Carew* and Michael Conley* (Department of Pharmacology, Duke University, 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 compare its anterograde properties with the autoradiographic (ARG) techniques used to demonstrate the anterograde transport of amino acids. We examined the retinal projection of the tree shrew by making intraocular injections of either 50 μl 30% HRP or 150-500 μl 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’, 5’-tetramethyl benzidine (TMB) and 3,3’ diamino-benzidine (DAB). 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, pretectal, etc.) as early as 8 hours post injection. The optimal results, however, were obtained after about 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. This and certain instances where the ARG method were superior to those of the ARG. For example, the ipsilateral projections to the pretectal complex and medial terminal nucleus were more distinct in the ARG 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 confirmed 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 retinal neuronal projections. The comparison of the sensitivity and the saving advantages of this method indicate that it 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 National Institute of Neurological and Communicative Disorders and Stroke RO1 NS 13202 and by National Institute of Neurological Diseases and Stroke NS 07850).
Isolation of Rapidly Synthesized Calcium Binding Protein from Symptomatic and Soluble Fractions of Rabbit Brain Cortex After Topical Application of 3H-Leucine. Zafar Iqbal and Sidney Ochs. Dept. Physiology and Medical Biophysics, Indiana University School of Medicine, Indianapolis, Ind. 46202.

In our earlier work the presence of a calcium binding protein (CBP) of 15,000 daltons was reported in rat brain synaptosomes (Iqbal & Ochs, J. Neurochem., 1978). The CBP was isolated from the symaptosome by means of a thermoregulator device controlling a heating lamp (200 mm/day). Upon arrival at nerve terminals the macromolecules turn over at different rates; of particular interest are two proteins. Similarly, when the synaptosomes or the synaptosomal soluble fraction obtained from synaptosomes after hypotonic shock was injected into animals, a significant amount of the 15,000 dalton protein continued to accumulate and available for transport to other nerve terminal glycoproteins.

In contrast to fucose, incorporation of [35S]sulfate into acid insoluble material in the retina was maximal at 2 h after which there was a rapid decline. The appearance of [35S]sulfate in ON, OT, LGB, and SC preceded by a short time that of the [3H]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 [35S]sulfate in the SC was equally divided between glycopeptides and glycosaminoglycans, indicating these macromolecules are transported at the same rate. Fractionation of labelled material on gels revealed a prominent radioactive doublet between 70,000 and 80,000 daltons at 3 h which was diminished at 4 h and absent at 8 h implying a 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 becomes available very soon after injection. Once transport is initiated, labelled macromolecules of both species are rapidly transported at a single rate (approx. 200 nm/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.


The axonal transport of [3H]fucose and [35S]sulfate-labelled macromolecules in retinal ganglion cells of the 16 day Sprague-Dawley rats was investigated following simultaneous intracellular injection of precursor. Maximal incorporation of fucose into acid insoluble material in the retina was achieved by 3 h after injection. Transported [3H]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, indicating the 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 90% of the labelled [35S]sulfate in the SC was in glycolipids and peptides. Proteins in the SC were fractionated on 7.5% SDS polyacrylamide gels. There were no radioactive peaks present by 24 h that were not present at 3 h. In the lateral geniculate body, the transport of [3H]fucose in the TO was slower and heterogeneous of fast transport rates for fucose-labelled material. However, over the 24 h period a 45,000 MW peak accounted for 93% of the radioactivity in the proteins of 40,000–180,000 daltons (second peak, lb) was 53.9% whereas the third peak, Ic, among other proteins a significant amount of the 15,000 dalton protein continued to accumulate and available for transport to other nerve terminal glycoproteins.


Horseshadish peroxidase (HRP, Sigma Type VI) 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 5 to 20 min. After 24 to 48 hr survival time, all animals were perfused intracardially with 1% formaldehyde and 2% 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 technique. Labelled neurons were identified and photographed under the light microscope. Results were analyzed by the means of the results from several sequential sections on copies of plates Extracted from the 60mm and Kippel 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 an ipsilateral anterior hypothalamic area. Movement in the caudal direction was also demonstrated. HRP-labeled neurons were also observed in the Nucleus accumbens, olfactory tubercle, septum; less than 12 percent of the prefrontal cortex was located in the adjacent nucleus accumbens, olfactory tubercle, septum; less than 12 percent of the prefrontal cortex was located in the adjacent nucleus accumbens, olfactory tubercle, septum; less than 12 percent of the prefrontal cortex was located in the adjacent nucleus accumbens, olfactory tubercle, septum; less than 12 percent of the prefrontal cortex was located in the adjacent nucleus accumbens, olfactory tubercle, septum. Some sections were processed with DAB tetra HCl for the brown reaction, and others with BDA for the blue reaction according to the technique.

Even though the clinically important amino acid phenylalanine (PA) is necessary for the proper functioning of the nervous system, it is not known how this amino acid is transported in axons. It is evident from the neurological effects of PA deficiencies or excesses, very little is known about the importance of this amino acid with respect to neuronal function. Our studies show that 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 branchial neuron such as that seen in the sensory ganglia where a length of fiber can be easily separated and divided for analysis. (U-14C)Phenylalanine (400 mc/millie. mole) was injected into the seventh lumbar dorsal root, and at various times up to 3 mm proximal to the injection site and the counts in the sciatic nerve advancing were examined under dark-field illumination for HRP reaction product visualized by autoradiographic techniques that, in addition to this, characterize the peripheral components of the vagus nerve and illustrates the transperikaryal passage of HRP across the Nodose Ganglion. Some of these fibers joined labeled neurons in NA. The central processes of NG neurons reach the lateral side of the medulla. These fibers were directed at DMN. Some of these axons joined labeled neurons of the DMN and some other axons looped ventrolat. The terminal axons were directed at the DMN. Some of these fibers seem to form terminal patterns in the components of the Nucleus of Tractus Solitarius (HPOS) such as the subnucleus gelatinosa of NTS. Such terminal axons also seem to be present within the DMN. Thus, this procedure enables the demonstration of all consistent and effective differences that exist between nerves and terminals. Retrogradely labeled neurons were seen in: 1) Dorsal Motor Nucleus (DMN) of vagus; 2) Nucleus Ambiguos (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 axons were seen just lateral to the central canal and medially in the ventral horn. HRP labeled fibers were seen entering through the dorsal root and descending. Axons could be classified into two groups, one belonging to axons of labeled neurons and the other belonging to the central processes of ganglia. Giallionc, interaxonal and neuronal transport of HRP could be demonstrated. This suggests that the two enzymes are axonally transported in a fashion distinct from that in ganglia. The number of [3H]proteins undergoing fast axonal transport in primary afferent nerves of different species seems to be higher. However, when nerve trunks are incubated in medium with calcium, medullary axonal transport is inhibited. This is probably due to the presence of calcium in the medium. Calcium is required in the soma for initiation of transport is therefore not affected by the finding of a calcium requirement for initiation of transport in the peripheral components of the vagus nerve and illustrates the transperikaryal passage of HRP across the Nodose Ganglion with the use of histochemical techniques.
100 EFFECTS OF COLCHICINE AND CYTOCHALASIN B ON AXONAL TRANSPORT IN NODRUGERINE 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 [3H] leucine are transported in four waves in the ascending norenergic 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 [3H] fucose labelled glycoproteins. Neuropeptide (NE) is also transported at a rate of 48 mm/d. Waves III (13-29 mm/d) and IV (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 made for changes in precursor incorporation) of [3H] proteins, glycoproteins and [3H] 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 microtubules 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.B. 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 NGF and the uptake of [125I]-NGF in the ciliary ganglion of 1-2 day post-hatching chicks following unilateral intraocular injection of I-NGF. The difference in radioactivity between ganglia on injected (I) and un.injected (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 [1-14C]-labelled taurine toxin, wheat germ agglutinin (WGA), and cytochrome C. Tetanus toxin and WGA have been shown to be transported in all peripheral and central neuronal systems but not 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 taurine 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 held views, NGF may have a physiological function in the parasympathetic nervous system.

102 QUABAIN INHIBITION OF FAST AXOPLASMIC TRANSPORT: Robert O'Neill,* G. D. 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 on axial transport of neurotransmitter and intracellular proteins, taurine, sodium and calcium. Ouabain at low concentration, has no effect on tubulin/microtubule polymerization in vitro, structures believed to be involved in FAXT. In FAXT of proteins and right vagus nerve was studied in vitro. [3H]-leucine was microinjected into the nodule 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 radioactivity 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 changes: loss of intracellular and interaxonal 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 NIHMO-02528, U.S.P.H.S.

103 AXONAL TRANSPORT OF 35S TAUrine ALONG NEONatal AND YOUNG ADULT RAT OPTIC AXONS. Michael Politis and Nicholas Ingoglia Dept. Physiol. and Neurosci., N.Y. 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 acid amino acid is axonally transported along goldfish optic nerves. In the present experiments the axonal transport of taurine was 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 postnataally. The activity of the catecholaminergic neurons of the ciliary ganglia was determined in relation to the optic nerve. Autoradiographic studies support the biochemical evidence for selective retrograde axonal transport of taurine in the ciliary ganglion. Thus, in contrast to held views, taurine may have a physiological function in the parasympathetic nervous system.

104 AUTORADIOGRAPHY OF NEUROTRANSMITTERS IN THE CAT. Fred Samson and J. Alejandro Donoso. Ralph L. Smith Research Center, University, CH 4056 Basel, Switzerland.

Autoradiographic studies support the biochemical evidence for selective retrograde axonal transport of NGF in the ciliary ganglion. Thus, in contrast to held views, NGF may have a physiological function in the parasympathetic nervous system.
SPECIFIC AND RAPID TRANSPORT OF FREE GLYCINE IN IDENTIFIED AXONS OF APLYSIA

C.H. Price, B.J. McAdoo, and G. Far.* Marine Biomedical Unit, University of California, Santa Barbara, CA 93106.

Neurons R3-R14 in the parieto visceral ganglion (PVG) of Aplysia may be glycinenergic (Price, et al., and McAdoo, et al., 1978 Brain Res. in press). Their axonal transport between nerve cell bodies and peripheral terminals is rapid and directed. After incubations for several hours, the nerve was either frozen intact or sliced into 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 R-amino acids were used instead of glycine, the axons of R3-R14 were labeled equally to other axons. At least 85% of transported radioactivity co-located with free glycine in thin-layer chromatographic analyses of pieces of nerve from experiments in which ganglia were incubated in media with 3H-glucose 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 (10 times as much). In the left pleuro visceral connective (which contains no R3-R14 axons), glycine was transported at 25 mm/day. Transport of glycine down the branchial nerve was inhibited by monovalent cations (HCl, NaCl, or KCl). By contrast, 2,5-Hexanediol (2.5H,) and 2,4-DNPH (2 mM), NaCN (10 µM), or high K+ (150 mM) did not inhibit transport. Proximal accumulation of radioactivity was observed. Glycine transport was retrogradely at a rate comparable to orthograde transport but in quantities 9 times less. In autoradiographs of nerve fibers 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 nonmetabolic role for glycine in these neurons, perhaps as a neurotransmitter. This work was supported by an NIH fellowship to CFH (5F32NS 038555) and NEHRM grant R3131 to DM.

DIFFERENTIAL TURNOVER OF AXONALLY TRANSPORTED GLYCOPEPTIDES.


It has previously been shown that axonally transported glycoproteins reach maximum accumulation in the goldfish optic tectum at 25-30 h after intracocular injection of 3H-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 of 20-30 days. In the present study, the distribution and turnover of axonally transported glycoproteins was assessed in the membranous and soluble fractions of goldfish tectum at various times after intracocular injection of 3H-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 in 20 days. In contrast, the 100,000 g soluble fraction showed a more rapid turn-over, with an apparent half-life 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-dialyzable 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 proteins in the nerve terminals. The various sized glycopeptides from the soluble fraction appear to have similar turnover rates while glycopeptides from membra nous fraction undergoes turnover in days. These patterns support the concept of differential utilization of various classes of glycoproteins at the nerve terminal.

A multiwire proportional chamber was used to detect axonal transport of 35S-methionine and 32P-phosphate labelled material.

R.E. Snyder, T.B. 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 of 35S-methionine and 32P-phosphate labelled material. Axonal transport in a motor nerve of the crayfish was studied using preparations from Xanthopus leucurus. Nerve cell bodies exposed to 35Smethionine were shielded from the detector by a lead-lined compartment; the sciatic nerves were placed in a chamber over the detector. In experiments with 32P, 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 35S-methionine experiments, the radioactive activity 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. Maximum label was transported at a similar rapid velocity. An order of magnitude less label was transported if 32P-phosphate was used in conjunction with phosphate-buffered saline than when the saline were HEPES-buffered and free of cold phosphate. With HEPES buffer, the amount of 32P transported was about two orders of magnitude less than the amount of 35S-methionine which was transported in similar preparations.

Transneuronal transfer (TT) of axonally transported protein-bound radioactivity was examined in the visual system of neonatal and adult hamster hamsters in vivo and in vitro. Intracranial 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, and a subsequent transient transport to LGB nerve endings in layers IV of visual cortex where it can be detected by scintillation counting or autoradiography. Non-specific TT from SC to overlying retinopetal cortex was also observed. TT was evaluated both 1 and 11 days after intracranial 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 retinopetal 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 (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 polypeptide 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 Laurow (Exp. Neurol. 39:44, 1974) 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).

Comparison of fast transported protein and glycoprotein down-flow patterns in sensory and motor fibers of hamster 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 transported in nerve fibers by an axoplasmic transport, primarily to the axon terminals, while the bulk of the proteins synthesized in the soma are transported more slowly. We compared the ontogeny, characteristics of fast transported 3H-fucose and 3H-glucosamine labeled glycoproteins with 3H-leucine labeled proteins in the motor and sensory fibers of cat sciatic after in vitro exposure to the labeled precursors. At 7 dorsal root ganglion or L7 ventral horn, the resulting outflow patterns showed a higher amount of incorporated 3H-leucine transported in the ventral than in 3H-fucose labeled components in the sensory fibers. The ganglion pool - nerve crest amplitude relationship (Ochs, J. Physiol. 255:249, 1975) was the same with either 3H-fucose or 3H-leucine as the precursor. The slope of the advancing front of 3H-fucose labeled activity was shallower than that of the 3H-leucine labeled proteins in the sensory fibers after 3 and 7 hours of downflow. A shallow front slope was also observed with 3H-glucosamine labeled material in sensory fibers. The maintained shallow slope of the labeled fronts in the sensory fibers with distance of the 3H-fucose labeled substance suggests, that in contrast to 3H-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 3H-fucose compared to 3H-leucine may be attributable to this process. The outflow patterns in the motor fibers generally show a somewhat higher amount of 3H-fucose labeled material transported than 3H-leucine labeled components, as judged by their crest amplitudes. In contrast to the sensory fibers, the slopes of the fronts of both 3H-fucose and 3H-leucine labeled glycoproteins and 3H-leucine labeled proteins were shallow in the motor fibers and this pattern was maintained after both 3 and 7 hours of downflow. This observation suggests that 3H-leucine polypeptides are processed in motor neurons at a slower initial rate than in sensory neurons. The 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 glycoprotein synthesis. Supported in part by NIH grant BRS 901 NS 8706-09 and NSF grant BNS 75-03688-A03.

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. 33192.

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

Two-dimensional gel electrophoresis of high-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 (Black & Lasek, Soc. Neurosci. 3:29, 1977). The hypothesis that ionophoresis that each component contains a distinct class of proteins was tested by SDS-PAGE analysis. 3H-lys and -pro were injected into the vitreous humour of guinea pig eyes and the animals sacrificed after 6 or 12 hrs for FC, 6 days for SC, and 38 days for SC. For FC, the optic nerve was cut slightly anterior to the chiasm to cause the FC axons to accumulate in a 3-mm segment proximal to the ligature was subjected to SDS-PAGE analysis. 3H-lys and -pro were injected into the vitreous humour of guinea pig eyes and the animals sacrificed after 6 or 12 hrs for FC, 6 days for SC, and 38 days for SC. For FC, the optic nerve was cut slightly anterior to the chiasm to cause the FC axons to accumulate in a 3-mm segment proximal to the ligature. The resulting outflow patterns showed a higher amount of incorporated 3H-leucine transported in the ventral than in 3H-fucose labeled components in the sensory fibers. The ganglion pool - nerve crest amplitude relationship (Ochs, J. Physiol. 255:249, 1975) was the same with either 3H-fucose or 3H-leucine as the precursor. The slope of the advancing front of 3H-fucose labeled activity was shallower than that of the 3H-leucine labeled proteins in the sensory fibers after 3 and 7 hours of downflow. A shallow front slope was also observed with 3H-glucosamine labeled material in sensory fibers. The maintained shallow slope of the labeled fronts in the sensory fibers with distance of the 3H-fucose labeled substance suggests, that in contrast to 3H-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 3H-fucose compared to 3H-leucine may be attributable to this process. The outflow patterns in the motor fibers generally show a somewhat higher amount of 3H-fucose labeled material transported than 3H-leucine labeled components, as judged by their crest amplitudes. In contrast to the sensory fibers, the slopes of the fronts of both 3H-fucose and 3H-leucine labeled glycoproteins and 3H-leucine labeled proteins were shallow in the motor fibers and this pattern was maintained after both 3 and 7 hours of downflow. This observation suggests that 3H-leucine polypeptides are processed in motor neurons at a slower initial rate than in sensory neurons. The 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 glycoprotein synthesis. Supported in part by NIH grant NS12393 and a biomedical research support grant. GCS is a NIH postdoctoral trainee (NS07904).

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 transport protein is rapidly transported in various neuronal systems. The axon contains major waves of radioactivity defined by their velocities: the fast component (FC), >250 mm/day; slow component b (SCb), 2.0 mm/day; and slow component a (SCa), 0.3 mm/day (Black & Lasek, Soc. Neurosci. 3:29, 1977). The hypothesis that these waves represent a specific transport appears as three major waves of radioactivity defined by their velocities: the fast component (FC), >250 mm/day; slow component b (SCb), 2.0 mm/day; and slow component a (SCa), 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. 3H-lys and -pro were injected into the vitreous humour of guinea pig eyes and the animals sacrificed after 6 hrs for FC, 6 days for SC, and 38 days for SC. For FC, the optic nerve was cut slightly anterior to the chiasm to cause the FC axons to accumulate in a 3-mm segment proximal to the ligature. The resulting outflow patterns showed a higher amount of incorporated 3H-leucine transported in the ventral than in 3H-fucose labeled components in the sensory fibers. The ganglion pool - nerve crest amplitude relationship (Ochs, J. Physiol. 255:249, 1975) was the same with either 3H-fucose or 3H-leucine as the precursor. The slope of the advancing front of 3H-fucose labeled activity was shallower than that of the 3H-leucine labeled proteins in the sensory fibers after 3 and 7 hours of downflow. A shallow front slope was also observed with 3H-glucosamine labeled material in sensory fibers. The maintained shallow slope of the labeled fronts in the sensory fibers with distance of the 3H-fucose labeled substance suggests, that in contrast to 3H-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 3H-fucose compared to 3H-leucine may be attributable to this process. The outflow patterns in the motor fibers generally show a somewhat higher amount of 3H-fucose labeled material transported than 3H-leucine labeled components, as judged by their crest amplitudes. In contrast to the sensory fibers, the slopes of the fronts of both 3H-fucose and 3H-leucine labeled glycoproteins and 3H-leucine labeled proteins were shallow in the motor fibers and this pattern was maintained after both 3 and 7 hours of downflow. This observation suggests that 3H-leucine polypeptides are processed in motor neurons at a slower initial rate than in sensory neurons. The 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 glycoprotein synthesis. Supported in part by NIH grant BRS 901 NS 8706-09 and NSF grant BNS 75-03688-A03.

Thirty-six female rats received injections of tritiated proline (100 uCi 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 tritum 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 rate, 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.

SUBCELLULAR LOCALIZATION OF PARTICLES INVOLVED IN THE TRANS­LOCALIZATION OF PROTEINS IN CEREBRAL TISSUE. Frederic P. White. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. A1B 3V6

The translational efficiency of newly synthesized protein from one subcelluar 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 [3H] leucine while other slices were incubated in 14C leucine alone. The slices were homogenized together and fractionated by differential sedimentation and discontinuous sucrose gradient techniques. Comparisons of the ratios of 3H/14C 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, P19. 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 great enrichment for an import particle has been obtained from the crude mitochondrial pellet. It [P2] has a density between 1.092 and 1.136, 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 P16 was shown to decrease in specific activity during the chase period while P22 increased in specific activity during the same period. Thus the conclusion drawn from the vinblastine experiments, that P16 was an exporter of protein and P22 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 some of the particles can be isolated by ultracentrifugation techniques which are involved in the vinblastine and Ca++ sensitive transport of proteins. This research was supported by NSC Grant MA-5404.
BASAL GANGLIA
RESPONSES OF CAUDATE NEURONS IN AWAKE MONKEYS TO A VISUAL STIMULUS THAT INITIATES A MOTOR TASK. J. Wayne Aldridge, R.J. Anderson and J.S. Murphy. Dept. Physiol., Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Monkeys were trained to perform a motor task involving wrist movement. 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 manipulation of 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 (65%) altered their cell firing with the appearance of the display. Ninety-two cells were inhibited, 56 were excited and 9 cells had both excitation and inhibition. The majority of the responses were brief and phasic in nature. The mean latencies were 260±64 msec for excitations and inhibitions respectively. Ninety-two cells (92%) responded before the onset of movement. The mean latencies were 200±07 msec for excitations 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.


Recently, increasing concern has been focused on the systems underlying spontaneous locomotor activity in hopes of better understanding the motoric dysfunctions responisible for hyperactive behavior exhibited by children. The exact neuroanatomical structures and neurophysiological functions responsible 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 serotoninergic cell bodies. In fact, lesions of nucleus linearis centralis alone were found sufficient to induce hyperactivity. These data suggest that serotoninergic as well as dopaminergic systems may contribute to the increased activity produced by lesions of the ventral mesencephalic tegmentum of the rat.


The existence of an interaction between dopamine (DA) and ACCh in the caudate nucleus has been accepted for many years. However, the exact nature of this interaction or modulation of ACCh activity by DA, i.e. whether DA has an excitatory or inhibitory effect on ACCh, is still a controversial issue. 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 forebrain stimulation comb which branched extensively to form a fine axonal plexus within and to some extent beyond, the doprinitic domain of the parent cell. Without branching 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, after giving rise to a few collaterals in the nucleus, continued into the internal capsule. Several of these latter axons were traced to a point near the entopudonuclear nucleus; however, the injection of HRP into this nucleus 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 NS0045.)


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 with 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). Monkeys received bilateral Cd lesions (200-400mg) were anesthetized with urethane (120mg/kg). SN stimuli (0.05-0.1 msec pulses) were applied through stereotactically positioned insulated needles. Neurons filled with HRP who were traversed by 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 6.3 msec. Often the EPSPs were followed by hyperpolarization lasting 100 msec or more. The injected neurons were located in various regions of the SN. The Cd-NA and Cd-NA-P areas were located 250-400 um anteriorly, 300-350 um dorsocentrally and 150-200 um mediolaterally. The substantia nigra have resulted in increased locomotor activity. Bilateral lesions of nucleus linearis centralis alone were found sufficient to induce hyperactivity. These data suggest that serotoninergic as well as dopaminergic systems may contribute to the increased activity produced by lesions of the ventral mesencephalic tegmentum of the rat.

Simple neurons in the subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr) and pars compacta (SNpc) were studied in the monkey during the performance of a step and a simulated tracking task (see Georgopoulo, A.P., and DeLong, M.R. The globus pallidus of the monkey: Neuronal activity in relation to movement, this volume). 227 neurons in both structures were identified penetrations in two hemispheres: 123 from STN, 90 from SNr 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, using 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 reach of the task. Most STN units were related to a distinct pattern of spontaneous discharge. Many cells were strikingly modulated by active movements of individual contralateral limbs, or even the face. These units related to the STN were found largely in the lateral portion of STN, where units related to specific body parts were grouped together supporting a somatotopic organization. Most units related to the medial part of STN were not clearly affected by movements. SNpc units had low spontaneous discharge rates and did not show clear modulation by movement. SNr units had high spontaneous activity, similar to those of internal pallidal units. Many SNr cells were modulated by movement, the or the firing of some units was increased or decreased as the monkey moved its limbs, and a few were modulated by eye movements.

It appears that the pars reticulata of the substantia nigra and the pallidum vary independently in response to external or internal stimuli, which may have a functional entity which has been divided by the internal capsula. This is suggested by: (a) the striking similarities in the morphology of the frontal and temporal segments of the internal pallidum, (b) the absence of the SNr, as in their afferent and efferent connections, (b) the nearly identical patterns of spontaneous discharge in the GPi and SNr, and the appearance of anterograde and retrograde heterotopic lamination into the GPi and SNr. However, the neural activity in the GPi and SNr, indicating further the arbitrarians' their separation by the internal capsula.


Most of the neurons in the monkey neostriatum are of medium size and, as shown in Golgi impregnation, includes cells with spiny dendrites and large somata (spiny I) and cells with smooth dendrites and small somata (aspiyn I) neurons are a key element of the basal ganglia.

The distribution of acetylcholinesterase (AChE) in some extrapyramidal nuclei was examined by means of intrastriatal injec-tions of kainic acid. The results showed that AChE-containing neurons in the striatum were among those which were destroyed by kainic acid. In complementary biochemical studies, it was determined that 90% of the total AChE activity in the striatum was localized in these AChE-containing neurons. Intrastriatal injections of kainic acid produced significant decreases in the activity of the glutamic acid decarboxylase in the substantia nigra, thus demonstrating that neurons contributing to the striato- and pallido-nigral pathways had been lesioned. However, we found that nigral AChE activity was 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 fibers proximal to the injection. In contrast, injections of colchicine into the nigro-striatal projection led to a proximal accumulation of AChE in the fibers 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.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Control value ± SEM</th>
<th>AChE</th>
<th>CAT</th>
<th>TH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>43.4 ± 7.09 umole/mg prot-hr</td>
<td>10.9 ± 0.41 nmole/mg prot-hr</td>
<td>8.1 ± 0.31 nmole/mg prot-hr</td>
</tr>
<tr>
<td>AChE</td>
<td></td>
<td>62.5 ± 3.05 umole/mg prot-hr</td>
<td>20.9 ± 1.23 nmole/mg prot-hr</td>
<td>10.8 ± 1.23 nmole/mg prot-hr</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td>104.0 ± 9.8% 16.7 ± 0.96 nmole/mg prot-hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td></td>
<td>93.9 ± 3.05% 7.88 ± 0.319 nmole/mg prot-hr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 involving the nigro-striatal bundle in the lateral hypothalamus (MFB lesions) of non-sedentary rats decreased 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, 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 > 2 weeks post-lesion compared to those tested at 7 days or from intact controls. In the caudate contralateral to the lesion, a gradual slowing of spontaneous firing was observed. The hallmark of this slowing was a shift in the spontaneous firing towards lower rates and longer inter-spike intervals. These effects were more evident in the contralateral caudate and contralateral substantia nigra than in the ipsilateral caudate or substantia nigra. A similar slowing of spontaneous firing was observed to occur in the contralateral subthalamic nucleus. These findings are consistent with an increase in the expression of monoamine oxidase activity in the contralateral caudate and substantia nigra following the MFB lesions. The net result of this remodeling is to produce a side-to-side asymmetry in the activity of caudate neurons. Preliminary studies have established that this asymmetry is modulated by a neuroactive factor in the striatum, but the source of this factor is unknown. Moreover, the source of this factor may be the substantia nigra. The role of this factor in the substantia nigra and the source of this factor is currently being determined. Further observations were made by using a post-lesion conditioned response technique where the monkey was rewarded with liquid or food for successful acquisition and maintenance of the task. Data for spike and behavioral events, and movement parameters (position, velocity and acceleration of the monkey) were collected for each movement. EMG activity from 16 arm, neck and paraspinal muscles was also collected during the performance of the task.

125 CAUDATE UNIT ACTIVITY IN RESPONSE TO INPUT FROM NUCLEUS CENTRALS MEDIALIS IN CATS. Roger L. Gastegi, Irma Zarco-Coronado* and Hector Brust-Carmona. Depto. de Fisiologia, 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. Single unit studies in cats (Diez-Martinez et al., Physiol. Behav. 19: 269-276, 1977) revealed projection of the nucleus centrales medialis (NCM) to the CN. To further study these projections the coordinate and localizing unit activity in the head of CN (A16) in response to input from NCM in normal and in cats with substantia nigral lesions were recorded for surgical injection of 6-hydroxydopamine. Extracellular recordings of unit discharges and their associated slow waves were recorded from stainless steel microelectrodes while stimulating NCM through concentric bipolar stainless steel electrodes. Electrical stimulation of NCM causes driving of some units in all three areas with the most common response consisting of a sequence of early firing (10-40 msec) with inhibition (30-200 msec) and rebound discharge. A second frequent response is potentiation of discharge frequency which may last only during the initial period of tetanisation (1-2/sec) or be prolonged after tetanisation for more than a minute. During transient potentiation the positive slow waves show transient enhancement. With the exception of this transient effect no other changes were observed in the extracellular field potentials of postsynaptic potentials in NC for other cholinergic inputs (Buchwald et al., Exp. Neurol. 32: 311-323, 1973). These preliminary findings suggest a heterogeneous organization of the CN in contrast to the usual view of a homogeneous organization. 1. Visiting Professor on leave from the Sect. of Physiology, Div. of Biology and MBS Col. of Vet. Med., Cornell University, Ithaca, N.Y.


Single neurons in the external (GPe) and the internal (GPI) segments of the globus pallidus of the monkey were studied in a step and pursuit tracking task. The manipulandum was a sight-right eight-foot handle which the monkey could grasp and move along a horizontal path with minimal friction. The display consisted of two rows of 14 lamps each (10 in upper row and 4 in lower) which were placed at the upper and lower limit of the range of movement, and the lower position of the handle. After holding in a starting position for a variable period of time, step movements were initiated by pressing down a handle. Movement duration, while pursuit movements of different but constant velocities were obtained by activating sequentially adjacent lamps. The movements were rewarded with sucrose with the monkey in pursuit mode and maintenance of the target. Data for spike and behavioral events, and movement parameters (position, velocity and acceleration of the monkey) were collected for each movement. EMG activity from 16 arm, neck and paraspinal muscles was also collected during the 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 using a passive movement paradigm, where the monkey was passively moved while a handle which the monkey could grasp and move along a horizontal path with minimal friction. The display consisted of two rows of 14 lamps each (10 in upper row and 4 in lower) which were placed at the upper and lower limit of the range of movement, and the lower position of the handle. After holding in a starting position for a variable period of time, step movements were initiated by pressing down a handle. Movement duration, while pursuit movements of different but constant velocities were obtained by activating sequentially adjacent lamps. The movement was rewarded with sucrose with the monkey in pursuit mode and maintenance of the target. Data for spike and behavioral events, and movement parameters (position, velocity and acceleration of the monkey) were collected for each movement. EMG activity from 16 arm, neck and paraspinal muscles was also collected during the performance of the task.
Packing and circling in pigeons following disruption of a nigrostriatal pathway, a possible nigrostriatal homologue. Irving J. Goodman and Albert J. Azary**, Dept. of Psychology and Neurology, West Virginia University, Morgantown, WV 26506.

Apomorphine induced compulsive pecking may be significantly or critically biologically related (3-4 days) with bilateral destruction of the pigeon's dopaminergic-rich pallidostriatal augmentum (PA), a homologue of the mammalian caudate-putamen. The present study employed the same model and compared the eventual behavioral effects by focusing on dopaminergic PA afferents, which originate in and around the nucleus tegmenti pedunculo-pontinis, pars compacta (TPc) and the nigrostriatal (TPn) pathway, a possible nigrostriatal homologue. Stereotactic placement of unilateral electrolytic (DC, 2 mA/15 sec) or 6 hydroxydopamine (6-OHDA, 8 µg/ul saline) lesions were made in TPn or TPc or intrastriatally by thereotaxic injection over a 5 minute period and the needle kept in place an additional 5 minutes. 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, while in the present study was that TPc/AVT lesions would favor pecking and circling away from the lesioned side, based upon findings in the lateral lemniscus, nigrostriatal pathways, the accompanying explanation of supersensitivity of denervated caudate units. Considering sensitivity changes as a means of explaining the above findings, we believe that apparently denervated TPc pigeons would have 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 TPc cells is a consequence of dopamine depletion, then post-lesion results, then, just the opposite, reduced sensitivity to apomorphine stimulation is suggested. So sooner have we begun to see, for an avian homologue of the nigrostriatal pathway when turn up, perhaps, an important behavioral exception.


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 close 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 following intrastriatal injections of KA. In the present study, an attempt was made to quantify these abnormal involuntary movements (AIMs) both in terms of a classification of AIMs and in terms of a scoring system for quantifying the AIMs. The heterogeneity in staining described above provides a basis for the concept of a fundamental subdivision of the striatum in the human being, cat and monkey into at least partially segregated, histochromically distinct units. It is of some interest suggesting the "dopamine islands" observed by Tennyson et al. (1972) and Olson et al. (1972). The study of such relationships may be essential to understanding the significance of the dopaminergic systems.


It has been known for some time that neurotransmitters such as dopamine exist in dendrites. In a companion paper (McGeer, McGeer and Inmanne, Dendroaxonal Neurotransmission II), it has been shown by specific techniques that neuronal dendrites contain both dopamine and acetylcholine in nerve endings for dendritically released dopamine in SN and acetylcholine in the neostriatum. This paper presents ultrastructural evidence that dopaminergic nerve endings for dendritically released dopamine in SN and acetylcholine in neostriatal dendrites. Postsynaptic structures were carefully examined in cross sections in which the microsections were 42 unbuffered O4H and preembedding staining with 24 uranyl acetate. In the EN, round vesicle-like structures of 20 to 60 diameter were often seen attached to the inner surface of post-synaptic membranes. The origin of the vesicle-like dendritic structures is clear. However, following the administration of horseradish peroxidase in vivo, the enzyme appears to be localised to similar structures found in both anterior and retrograde transport. Since the enzyme is also concentrated in the smooth endoplasmic reticulum, it seems possible that these exist in both intracellular structures and are related. The idea that dopaminergic dendrites may be involved in neurotransmission data suggesting dendroaxonal transmission of dopamine and acetylcholine is supported by NSF grant BNS 75-1875P.


Defining characteristic of the mammalian stratum 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 Gomori and Silverman & Koenig.

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 highly localized, 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, 0.8mm in the human and 0.82139mm in the monkey. 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 frontal 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, these 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 50um sections) and human (75 75um sections). Important observations are first, that despite some abrupt changes in their shape from section to section, many of the above discontinuities are actually continuous with one another, forming elements of a highly branched three dimensional labyrinth. Furthermore, the enzyme content of areas continuous with one another was determined histochromically in the adult cat, monkey and human using the histochemical staining. It is an unresolved problem how the cholinesterase-poor compartments described here relate to other inhomogeneities in the dopamine system. This study has been particularly interesting instance being the "dopamine islands" observed by Tennyson et al. (1972) and Olson et al. (1972). This study of such relationships may be essential to understanding the significance of the dopaminergic system.
131 NONDOPAMINERGIC AND DOPAMINERGIC NIGROSTRIATAL PATHWAYS IN RATS.
John M. Hedeen, 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 lesions showed degenerating fibers in the ventral lateral septal area, where the dopaminergic nigrostriatal axons travel, but a group of degenerating axons is seen which separates from the efferent pathway and proceeds retrogradely through the thalamus along the internal capsule. The axons pass through the entopeduncular nucleus in the network of cellular areas and transversely oriented fiber bundles, then enter the paranigral ventral half of the globus pallidus and enter the neostriatum. Degenerating axons in the neostriatum are especially concentrated in the ventral lateral and ventral central half of the globus pallidus. The identification of this pathway as nondopaminergic, and its role or presence 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 temporal 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 strongly - their following a partly different course from the dopaminergic nigrostriatal pathway before entering the zona incerta. The demonstration of the nondopaminergic nigrostriatal pathway without interference from dopaminergic fibers.

The dopaminergic nigrostriatal pathway, as described by Ungerstedt (1971), is supported in autoradiographic cases after HRP injections of tritiated amino acids. The axons in this path traverse the ventral tegmental area before entering the zona incerta. Presumably, they remain terminal only by the lack of strong labeling, they follow a partly different course from the nondopaminergic nigrostriatal pathway, for example traversing the entopeduncular nucleus before entering the zona incerta. The nondopaminergic nigrostriatal pathway consists of collaterals of nigrostriatal dopaminergic cells by the striatonginal projection may not be responsible for the METH-induced TH depression. Likewise, reversal of TH depression by haloperidol and yAG 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 metabolic inhibition of TH activity are being explored in an effort to explain the METH-induced depression of striatal TH activity. (Supported by USPHS grants GM 00153 and DA 00889.)

132 PROJECTIONS OF CENTRUM MEDIANUM TO CAUDATE NUCLEUS IN THE CAT AS DEMONSTRATED BY RETROGRADE TRANSPORT OF HORSE RADISH PEROXIDASE.

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

133 NEURAL CONNECTIONS OF THE AVIAN LATERAL CORTICOID AREAS.

In pigeons, HRP was injected into the temporo-parieto-occipital area (TPo) and area corticoida dorsolateralis (CDL) of the telencephalon. Retrograde transport of HRP was observed to three ipsilateral cell fields: the hyperstriatum ventrale (Hv), pericortical 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 and AV resulted in labeling of cells in discrete fields of the frontal and caudal ventral neostriatum and neostriatal ventral nucleus. HRP labeled neurons ipsilaterally in the hippocampus (Hg), 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. The results demonstrate that at least some regions of CDL receive their thalamic input from CM rather than n. centralis ventralis; however, no clear boundaries or correspondence is 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.

134 LESIONS OF THE STRIATONIGRAL PATHWAY DO NOT REVERSE METHAMPHETAMINE-INDUCED TYROSI NE HYDOXYLASE DEPRESSION.

Adair Hotchkiss* and James M. Gibson*, L .M. Jarchow, Deps. Pharmacology, Univ. of Utah, Salt Lake City, UT 84132.

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

One type of lesion was produced by intrastral injection of kainic acid (2 µg in 1 µl) in rats weighing 230 to 250 g. Ten days later METH was administered (10 mg/kg, i.p.) every 6 h for 5 injections. Thirty-six hours 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 (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. 193:903, 1977). Preliminary data indicate equal TH depression in both sides (Buening and Gibb, 1969); y-aminobutyric acid (yAG, 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 and the substantia nigra. The results demonstrated that at least some regions of CDL receive their thalamic input from CM rather than n. centralis ventralis; however, no clear boundaries or correspondence is 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.


Nigro-neostriatal relationships were assessed by protein-incorporation autoradiography, horseradish peroxidase (HRP) histochemistry, and cytochrome oxidase histochemistry. In the terminal field of the nigro-neostriatal pathway, the nigro-neostriatal fibers with pars compacta dendrites projecting into pars reticulata, we used protein-incorporation autoradiography in combination with AChE histochemistry (see Butcher, 1976; in: Cholineric Mechanisms and Psychopharmacology; Jenden, Ed.; Plenum Press, New York).

Nigro-striatal fibers—containing both AChE and DA and traveling in the ventromedial mesencephalic tegumentum, field H4 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 soma 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 nigro-striatal pathway 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 this region.

Since HRP is transported in anterograde as well as retrograde directions the nigro-striatal injections also disclosed a topographic relationship between neurons originating in the motor cortex: the cell bodies of the nigro-striatal fibers were seen to extend past the anterior commissure, into the substantia nigra, pars reticulata and the HRP-containing somata of pars compacta apparently giving rise to the nigro-striatal tract (Horn and Adams, 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 Canada, Canada.]


The subcellular concentration of glutaminase in the symposi-omal fraction of brain (Bradford and Ward, Brain Res. 110, 115 (1976)) suggests that the enzyme is not an important part of the transmitter pools of glutamate, GABA, or GABAergic systems or that the latter can be destroyed by cortical lesions without effect on the glutaminase in KA-lesioned striata. The KA-lesioned striata was in the ventromedial mesencephalic tegumentum, field H2 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 soma of those target cells. Striato-nigral fibers, coursing prominently through the internal capsule, were observed to contact numerous AChE (DA)-containing den-


Recent anatomical evidence suggests that motor cortex projections in a somatotopically organized manner to the putamen, such that axons arising from the "arm" and "face" areas terminate in rostrocaudal, intermediate and ventromedial regions, respectively, of the putamen (H. Kühne, Brain Res. 1975). The observations that arm movements are mediated almost exclusively in intermediate regions of putamen (S. Liles, Fed. Proc. 1970) and units related to orofacial movements occur in ventromedial regions (Liles, unpublished observation) 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 utilizes the fine and detailed distribution of arm MR units within intermediate regions of putamen.

Monkeys trained to push or pull a manipandulum to obtain a food reinforcement (S. Liles, Fed. Proc. 1970) were prepaired for single unit recording after the technique of Evarts (1965). Electrode penetrations were made at an angle of 36° from vertical. Unit response magnitude was 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 units were all encountered over an axial distance of only 400-500 µm; during other penetrations three or four such clusters of MR units were found. NR 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 in the same site of each of these pairs of units showed similar NR 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 NR activity 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 NR properties of neurons in putamen. (Supported by USPHS Grant NS-00997 and the E.G. Schleider Foundation).


Rats that received injections of 6-hydroxydopamine (6-OH-DA) into the area ventralis tegmenti displayed a syndrome of impaired orienting (sensory inattention) characterized by decreased movement of the forepaws, loss of head-turning to visual stimuli and decreased latencies for head-turning to visual stimuli and decreased latencies for tactile or olfactory stimulation that was evident upon neurological examination. When the 6-OH-DA was injected unilaterally, these impairments were apparent upon neurological examination. When the 6-OH-DA was injected into intermediate regions of the putamen, but frequently occur in small neuronal colonies or clusters. The fact that neurons within these clusters show similar NR activity 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 NR properties of neurons in putamen. (Supported by USPHS Grant NS-00997 and the E.G. Schleider Foundation).
139 AN ALLEL OF DYSTONIA MUSCULARIS EXHIBITING LESIONS IN SOME AREAS OF THE EXTRAPRAMOTOR SYSTEM. Anne Messer, Norman L. Strominger and Lorraine Flaherty. Div. of Labs and Research, NYS Dept. of Health and the Department of Anatomy, Albany Medical College, Albany, NY 12201 (12208). An autonomic massive mutation, characterized by severe motor deficits, arose spontaneously in the BALB/cJ mouse stock maintained on the NYS Dept. of Health Labs. At about two weeks of age, the mutant mice showed bright movements, disorders and abnormal limb placements. The syndrome is steadily progressive to extreme ataxia with walking side-to-side movements, and some animals died the first week after birth. All mutants, whether killed or of normal age, died within 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 muscularis, an allelism test was performed. Crosses between strain 20 and a known non-syndrome from the two mutations are allelic. Therefore the mutation found here will be called dAlD. The pathology of dAlD is now being compared to that of Dm.

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 although they may not account for the entire clinical syndrome. The dAlD shows abnor­ malties in the red nucleus and in part of the striatum, in agreement to some degree with description described above. Formalin-fixed, paraffin-embedded serial sections (15 µ) stained with thionin show pathological changes in large coarse neurons from mice of the first generation that have been fully de­ veloped by four weeks and coordination actually improves slightly after a few months. Although sometimes smaller than littermates, mutants can survive many months.

140 ELECTRICAL ACTIVITY IN THE IN VITRO CAUDATE PREPARATION. J.J. Miller, B.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 the small lesions induced by picrotoxin could damage the whole preparation, an in vitro experiment was undertaken to examine the electrophysiological properties of a basal ganglia preparation in the absence of any potential components of the basal ganglia, the present investigation was undertaken to examine the electrophysiological properties of the in vitro caudate preparation. The rat caudate nucleus (500 µm in thickness) were maintained in a modified Ringer's solution at 34-36°C and superfused with 95% O2-5% CO2. Bipolar semi-micro stimulating electrodes were positioned at the convergence of the internal capsule or in the cellular zones between the medially oriented axon fascicles. Extracellular unit activity and spontaneous or 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. The stimulation site was thus tested to determine if single spikes were usually superimposed, was evoked at similar latencies. When a CA1-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 Co2+. The activation of sponta­ neously discharging neurons 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 150 usec. In CI deficient medium this inhibition was eliminated and a dramatic increase in the amplitude of the evoked population spike and background discharge rate was observed following a 20 usec paired pulse. This resulted in an enhanced test response at intervals of 10-100 msec.

Recovery of the control response was obtained when the CI con­ trolled the medium, with the cellular responses to the evoked responses have not been determined, these results provide preliminary evidence of the viability of the in vitro caudate slice as a suitable model for studies of the electrophysiological and ionic mechanism underlying synaptic transmission in this region.

Supported by the Medical Research Council.


The fiber projection from the caudate-putamen to the pallidum forms one of the two great effenter systems of the mammalian striatum. In order to study the distribution within the striatum of the fibers given rise to the strio-pallidal axons, we injected the retrograde tracer, horseradish peroxidase (HRP), into the pallidum in the cat. Two points of technique were dictated by our attempts to stain as nearly completely the cell-labeling of the striatum as possible: first, we injected very large amounts of HRP; and second, we used the BDA and TIM histochemical techniques of Mesulam to detect labeled neurons. We report here on findings in 3 cats in which large pallidal deposits produced extremely dense labeling of striatal neurons.

Most striatal neurons contain projections to both the head and to the tail of the caudate nucleus. The distribution of HRP-positive neurons was by no means uniform. Instead, in each case, a pattern of cell-labeling appeared in which large fields of labeled neurons were suddenly and repeatedly interrupted by variably shaped, roughly 0.5-2.0 areas containing variously few and often scarcely any labeled neurons. These sparsely labeled zones were easily visible with the naked eye in cross-sections through the head of the caudate nucleus. The HRP-positive neurons were found to be whorled nearly the full width of the caput (2-3mm), but were rounded or of complex shape. By tracing individual zones from section to section it became clear that in many instances in serially adjoining sections were continuous with one another.

The enzyme deposits were in all cases large, involving not only both segments of the caudate nucleus, but also the ventral and the anterolateral aspects of the striatum. In one case the substantia nigra (SN) itself was also labeled in the injected side. In no other cases in any of the animals could the possibility be excluded of infiltration of perifornical nigrostriatal fibers by the HRP. It is not yet clear, therefore, whether separate populations of neurons in different parts of the caudate's projection to GPe, GPi and SN, or whether the sparsely labeled zones here described represent neuronal populations with intrinsic striatal connections. Experience with 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 the small lesions induced by picrotoxin could damage the whole preparation, an in vitro experiment was undertaken to examine the electrophysiological properties of a basal ganglia preparation in the absence of any potential components of the basal ganglia, the present investigation was undertaken to examine the electrophysiological properties of the in vitro caudate preparation. The rat caudate nucleus (500 µm in thickness) were maintained in a modified Ringer's solution at 34-36°C and superfused with 95% O2-5% CO2. Bipolar semi-micro stimulating electrodes were positioned at the convergence of the internal capsule or in the cellular zones between the medially oriented axon fascicles. Extracellular unit activity and spontaneous or 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. The stimulation site was thus tested to determine if single spikes were usually superimposed, was evoked at similar latencies. When a CA1-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 Co2+. The activation of spontaneously discharging neurons 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 150 usec. In CI deficient medium this inhibition was eliminated and a dramatic increase in the amplitude of the evoked population spike and background discharge rate was observed following a 20 usec paired pulse. This resulted in an enhanced test response at intervals of 10-100 msec.

Recovery of the control response was obtained when the CI con­trolled the medium, with the cellular responses to the evoked responses have not been determined, these results provide preliminary evidence of the viability of the in vitro caudate slice as a suitable model for studies of the electrophysiological and ionic mechanism underlying synaptic transmission in this region.

Supported by the Medical Research Council.

142 A DESCENDING PATHWAY INVOLVING NIGRAL-INDUCED HEAD TURNING MOVEMENTS. Fereyeh 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. This study was undertaken to examine the electrophysiological and molecular nature of the nigral-induced head turning movements in rats with ipsilateral brain transections just anterior to SN in order to eliminate the effects of strato-nigral or corticofugal fibers which may affect SN.

Experiments were carried out in Sprague-Dawley rats (350-450 g) which were decerebrated. Two groups of rats were anesthetized and had a concentric bipolar electrode stereotaxi­ cally inserted into SN (pars reticulata, pr.) (A2.75, L2.3, D-1.5) (Skinner, May involved, W. T.). The other group were anesthetized and a transcranial lesion of the mesencephalon (A4.25, 10.9-4.35, D-2.2) just anterior to SN was performed. A bipolar stimulating electrode was then inserted into SN 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 (1mg/kg) administered in the lesioned group (Group II) did not abolish head turning in the non-lesioned group. However, haloperidol also raised the threshold to induce turning in some animals, but was found to lower threshold in others. The lowering of the stimulation threshold was observed in both lesioned and non-lesioned groups.

Recent neuroanatomical findings have defined a pathway from SNpr to the superior colliculus and midbrain tegmentum. In order to determine if the nigral stimulation induced head turning movements, animals of Group II were given a further lesion involving the ipsilateral superior colliculus (A0.85, L5.1, D1.5). Haloperidol was allowed to act for a few minutes after the lesion 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 to spinal cord motoneurons. (Supported by Dalston Research Center)
These experiments are part of a series of studies assessing the maturation of the 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 functionally mature in the first few weeks of life. We have now extended this work to show that the structures evoked action potentials from extracellularly recorded Cd neurons in 1 or 2 day old kittens. Extracellular recordings were obtained by inserting tungsten electrodes into the Cd of young kittens with a birth to 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 7% to 45% with age from about 73% to 45%.

Supported by USPHS grants HD-05958, MH-07097 and NS-12324.

In older kittens, the majority of responses were either purely inhibitory or involved excitatory-inhibitory sequences. About 40-50% of Cd responses were either excitatory only regardless of age. Of these, the percent responding with the same type of response decreased with age from about 73% to 45%.

The present experiments concern development of caudate outputs to the globus pallidus. Extracellular spike responses were evoked in Cd 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 even as early as 2 days of age. Latency of the 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.

In neonates, the percent of units responding to Cd increased from 7% to 45% with age from about 73% to 45%.

In older kittens, the majority of responses were either purely inhibitory or involved excitatory-inhibitory sequences. About 40-50% of Cd responses were either excitatory only regardless of age. Of these, the percent responding with the same type of response decreased with age from about 73% to 45%.

The present experiments concern development of caudate outputs to the globus pallidus. Extracellular spike responses were evoked in Cd 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 even as early as 2 days of age. Latency of the 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 7% to 45% with age from about 73% to 45%.

Supported by USPHS grants HD-05958, MH-07097 and NS-12324.

In neonates, the percent of units responding to Cd increased from 7% to 45% with age from about 73% to 45%.

In older kittens, the majority of responses were either purely inhibitory or involved excitatory-inhibitory sequences. About 40-50% of Cd responses were either excitatory only regardless of age. Of these, the percent responding with the same type of response decreased with age from about 73% to 45%.

The microstraital system was investigated by recording the intracellular responses of horseradish peroxidase (HRP)-identified substantia nigra (SN) and retrorubral (RR) neurons. These neurons were preferentially activated by stimulation of the caudate (Cd) nucleus and 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 placed stereotaxically in the head of the Cd nucleus and in the IC-MFB (A: 10, Reinoso-Suarez). Recording microelectrodes were filled with 2M MCI (30-50 megohms) or 4% HRP in 0.2 M MCI-Tris buffer (pH 7.6). Cd nucleus stimulation evoked IPSPs with latencies of 3-15 msec (x̅ = 7.9). IC stimulation produced mostly IPSPs (1.0-9.0 msec; x̅= 5.5) but occasionally these IPSPs were preceded by depolarizing potentials with latencies of 1.2-6.0 msec (x̅= 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 1-5 m/sec. Neurons identified by intracellular injections of HRP were located in pars compacta of SN and pars reticulata of RR and fusiform soma 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 collateralizing until terminating in complex thickenets at radial distances of 240-860 µm. Axons arose mainly from primary dendrites. For some neurons, axon coursing 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 NS04095.)


Acid-induced degeneration 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 from emotional disorders. Supported by the Medical Research Council of Canada.


Ascending influences of the brain-stem reticular formation are thought to distribute through the mesencephalic central tegmental field (FTC) and to disclose some control circuitry. By using the FTC as a landmark, we identify the target structures of rostrally projecting cells in the mesencephalic central tegmental field (FTC) and to disclose some control circuitry. We then provide evidence that FTC neurons are reciprocally connected to the zona incerta (ZI). The FTC and ZI are known to be part of a tegmental loop involved in the control of emotional reactions. To test the hypothesis that the upper reticular formation and related subsystems play a role in forebrain functions in the rat, we have performed autoradiographic studies of horseradish peroxidase (HRP) identified cholinergic and GABAergic neurons in the striatum. KA-treated rats have been used as an animal model of Huntington’s disease by Coyle & Schwarz (Nature 263:244, 1976). However, this analogy has not been supported by studies of the effects of KA on motor behavior.

In order to substantiate the electrophysiologic findings, the retrograde transport of HRP was used to identify the target structures of rostrally projecting cells in the mesencephalic central tegmental field (FTC) and to disclose some control circuitry. 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 chalamic 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 ZI terminals ending in ZI was positively answered in parallel experiments where unit discharges belonging to ZI cell bodies were monosynaptically elicited (1-3 ms) following focal stimulation in FTC. This midbrain reticular–Zona Incerta 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. The fibers arising in ZI terminal fields in FTC were identified with the diamino-benzidine method and the improved, tetramethyl-benzidine procedure of Neusament, J. Neurochem., 28, 106, 1979. Labelled neurons were found 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. These reciprocal connections 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 NRC grants MT-3689 and MT-5781).
Acute and long term effects of kainic acid (KA)-induced lesions used here destroy striatal neurons but leave intact nerve endings and fibres of passage. One week after unilateral striatal injections of 3 µmoles 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 µmoles KA and 10 µmoles KA reduced CSAD activity in the striatum to 94%, 38% and 27% respectively. Tau­ rine levels in the striatum showed a significant reduction to 64% of control levels after an injection of 5 µmoles of KA. A smaller but dose-dependent reduction in CSAD activity was also seen in the ipsilateral SW after intrastratal 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 toxic, the lesion following its injection gives 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 CSAD activities. Intrastratal 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 lesions 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.

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 neuroexcitatory and neurotoxic 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 by 5 mM unlabeled 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 pmol 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 ouabain was without effect. Also, the rate of the stratum 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 distinctly different from the glutamate receptor.

(3Supported by the Medical Research Council)
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, P1-N1. 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, P2-N2-P3. The P2 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

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 3H-dopa formed from 3H-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 33.3 ± 21.7 ng/mg 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 NE 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.

<table>
<thead>
<tr>
<th>Control</th>
<th>Carbidopa</th>
<th>Carbidopa + Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE (mg/12h)</td>
<td>427.3 ± 134.9</td>
<td>691.6 ± 112.1*</td>
</tr>
<tr>
<td>Tyr (100mg/kg)</td>
<td>231.3 ± 75.2</td>
<td>222.0 ± 73.7</td>
</tr>
</tbody>
</table>

*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 Juty. Dept. of Neuros., Loma Linda Univ., Loma Linda, CA, 92350 and Johnson Research Foundation, Univ. of Penn., 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 O2, (pO2), by 1) increasing the B.P. (2) increasing the F1O2, at Iast up to 60%, and 3) increasing the CBF by additional CO2 inhalation. These studies were carried out in a series of cats under N2O0 anesthesia, in a 2/1 ratio. They were based on the use of 25 u teflon coated platinum electrode inserted 1-2 mm in the prefrontal cortex to record pO2. In all cats it was necessary to exceed autoregulation of f1O2 or of B.P. by increasing the mean B.P. or F1O2, by a degree beyond 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 pO2 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,a3 (by dual beam, dual wavelength spectrophotometer), flavoprotein (by flying-spot fluorometer), and NADH (by fluorometric techniques). The present results show that carbidopa (100 mg/kg) administration enhances NE synthesis in (Science 185:183, 1974) and release from CNS noradrenergic neurons. The results of these ongoing animal studies into both canals influence. The data were then entered into a computer to determine the effect of treatment with VIL on cerebral pO2 and increase in oxidized state of members of the electron transport chain, offer a reasonable explanation for the acute improvement following microanastomosis.

The control K+ before the insult averaged 2.7 mM in normal cortex and 4.8 mM in glotic cortex. After the carotid arteries were clamped the K+ increased. In normal cortex the K+ rise was a complex exponential and resembled spreading depression. The maximum rate of rise averaged 3.53 mM/sec and the K+ reached a mean of 99 mM/L. The K+ rise in glotic cortex was linear and averaged 3.1 mM/min. The maximum K+ reached 26 mM before reversal of the insult.

The results were reversed by unclamping the carotids. The K+ fall was described by a single exponential function in both normal and glotic cortex. The rate constant averaged -0.25/sec in normal cortex and -0.004/sec in glotic cortex. Shoots of K+ below control levels of un to 1.2 mM (mean .65 mM) occurred in normal cortex but were not seen in glotic cortex. Since neurons and axons are absent in the glotic cortex, it is postulated that the presence of neurons and axons are necessary for the occurrence of spreading depolarization. The results of these experiments support the conclusion that the increase in pO2 and increase in oxidized state of members of the electron transport chain, offer a reasonable explanation for the acute improvement following microanastomosis.


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 in untreated PKU rats 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 phenylalanine into brain was impeded by VIL treatment of infants with PKU. 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 the FiO2 was increased. During this period on VIL serum Phe remained unchanged at 3174 uM, while CSF Phe decreased from 1049 to 882 uM. In a follow-up group of normal and mildly ischemic cats the results of a significant rise in pO2 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,a3 (by dual beam, dual wavelength spectrophotometer), flavoprotein (by flying-spot fluorometer), and NADH (by fluorometric techniques). The present results show that carbidopa (100 mg/kg) administration enhances NE synthesis in (Science 185:183, 1974) and release from CNS noradrenergic neurons. The results of these ongoing animal studies into both canals influence. The data were then entered into a computer to determine the effect of treatment with VIL on cerebral pO2 and increase in oxidized state of members of the electron transport chain, offer a reasonable explanation for the acute improvement following microanastomosis.
ROLE OF ASPARTIC AND GLUTAMIC ACIDS IN THE ATAXIA PRODUCED BY THIAMINE DEFICIENCY. Roger F. Butterworth, Dillie Hanx and Andrée 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, glutamic acid, aspartic acid, glutamic and taurine were measured by a double-labeling 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 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 aspartic and glutamic 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 thiamin in membrane excitability will be discussed.

Supported by grants from Université de Montreal CAIRF and The Association Canadienne de l’Ataxie de Friedreich.

SYNTHESIS OF MYELIN-ASSOCIATED GLYCOPROTEINS IN PROTEIN-DEFICIENT RATS. Mary J. Druse Manteuffel and Nancy L. Kretz, 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 or protein-deficient diets (8% protein) during the first 20 days after parturition. A double-label isolation technique was used whereby a control rat was given an intraperitoneal injection of 14C-fucose 18 hours prior to sacrifice and a protein-deficient rat was given fucose babeled with the isotope. Brain homogenates from a control and protein-deficient pup were combined. Purified myelin (JNC 21: 745, 1973) was subfractionated (BBA 329: 305, 1973) into light, medium and heavy myelin. 14C radioactivity was determined in percent casein content (%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, VMp of the thalamus, and motor cortex, with somewhat less difference in VPL, VL and medial globus pallidus. With extension to contralateral face and hindlimb 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 350% in lateral globus pallidus, twice that of any other structure. (Supported by NICHHD Grant HD06364.)


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 electroencephalograph and electromyography. An ear-clip electrode. In order to evaluate the generality of that finding, we tested the susceptibility of malnourished and normal rats to pentylentetrazol (PTZ) induced and kindled motor seizures. 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 electroconvulsive shock applied via electrolytic electrodes. In order to obtain evidence for the generality of that finding, we tested the susceptibility of malnourished and normal rats to pentylentetrazol (PTZ) induced and kindled motor seizures. 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 electrolytic electrodes.

The effect of chronic malnutrition on the cerebrovascular endothelium was studied in anesthetized rats using two methods: (1) determination of cerebral blood flow by radioactive microspheres and (2) calculation of cerebral blood flow using a semi-quantitative method of autoradiography. Using these methods with those of the isotope in arterial blood, the actual rate of focal 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 bi-laterally 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, VMp of the thalamus, and motor cortex, with somewhat less difference in VPL, VL and medial globus pallidus. With extension to contralateral face and hindlimb 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 350% in lateral globus pallidus, twice that of any other structure.
164 PIAL ARTERY PRESSURE IN THE DOG: EVIDENCE THAT AUTONOMIC INNERVATION DOES NOT AFFECT SURFACE BRAIN ARTERIES.
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. 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. Plaey 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 support the hypothesis 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 ISCHEMIA.
Kyuya Kogure, Hiroshi Morooka*, Raul Busto* and Elena Martinez*, Cerebral Vascular Disease Research Center, Univ. of Miami, Miami, FL 33132.
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 more severe disturbance of brain development have not been reported. In studies using a rat model to determine the impact of 9E protein diets, with normal caloric intake, 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% increase in the perimeter of the ER profile per unit area of dendritic section.

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

This alteration in the ultrastructure of Purkinje cells may be due to factors extrinsic to the Purkinje cell such as fewer granule cells and fewer processes of presynaptic neurons which are reduced in size. Another possibility is that the deficiency may act intracellulary by interfering with the Purkinje cells' ability to produce a cytoskeleton of tubules and filaments which are necessary for the maturation of a sizeable neuron. In either case, the excess ER may result since the dendritic trees are small. (Supported by the USPHS Grant HD-10936).

166 A TEST OF AUTO-OXIDATION OF PARTIALLY DISRUPTED BRAIN TISSUE IN VITRO.
Kooyu Kogure, Hiroshi Morooka, Raul Busto and Elena Martinez*. Cerebral Vascular Disease Research Center, Univ. of Miami, Miami, FL 33132.
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 initial injury by mechanical insult, restoration of oxygen may facilitate lipid peroxide production and catabolization of subcellular components. Three groups of rhesus monkeys were adjoined to each other by dense particles between the cyto-
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 ischemia. The brain samples were then assayed for energy metabolites, and the free radical reaction indices, melonol 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. 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) especially oxygen to the injured brain cell initiates free radical reactions.

167 ROLE OF ERYTHROCYTE CARBONIC ANHYDRASE I IN OXYGEN DELIVERY TO BRAIN.
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 and temporal relationship between the unloading of oxygen from hemoglobin in the tissue 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 release of CO₂ from hemoglobin is somewhere between 1 and 5 seconds. Only 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 CO₂ to bicarbonate (HCO₃⁻). Acetazolamide, a potent inhibitor of carbonic anhydrase, upon cerebral oxygen consumption (CMRO₂) in lightly anesthetized, passively ventilated rhesus monkeys. Cerebral blood flow (CBF) and CMRO₂ were measured with oxygen-15 labeled water and oxygen-15 labeled bicarbonate. Measurements were made in CBF and CMRO₂ during the infusion of acetazolamide (30 mg/kg). Acetazolamide produced a 32% decrease in CMRO₂ from 4.16 to 2.82 ml/min/100 g. The decrease in CMRO₂ was associated with a 52% decrease in CBF.

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; NS-6833 and HD-11851.

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). To determine if these changes persisted into adulthood, animals were cross-fostered at birth to dams of the same diet (25% casein). These alterations were correlated with increased T3 transport. Conclusions: (1) alterations in thyroid hormone transport: Km = 2.7 nM for T4. Transport of iodine across the blood-brain barrier early after a cold lesion. This data demonstrates that 18F-fluorodeoxyglucose can be utilized in both left and right occipital cortices from a small light located at its center. This light randomly dimmed in man. Alterations in functional activity change the metabolic ratio of the involved regions of the brain which can be detected with 18F-fluorodeoxyglucose. 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 blinded. The occipital cortex was symmetrically labeled bilaterally in both tests, but in the blinded 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 stimulated with high contrast black and white line and dot stimuli 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 visual field decreasing the metabolic ratio, which is presumably due to visual stimulation of the left visual field. In the somatosensory study, rapid brush stroking of the fingers with the light right showed a significant decrease in glucose utilization early and in the left postcentral gyrus at tomographic levels 0M-8 cm and 0M-9 cm. The cortical region corresponding to the finger and hand area is described by Penfield and Rasmussen (1950). This asymmetry was not seen in control subjects without somatosensory stimulation. These studies demonstrate that 18F-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)

SOCIETY FOR NEUROSCIENCE

168

169

170

171

180
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 cereveaux 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).
TOPOGRAPHY OF AFFERENT BRAIN STEM PROJECTIONS TO POSTERIOR VERMAL CEREBELLM OF THE RAT. S. Ausim Azizi*, Richard A. Burme and Donald J. Woodward (SPCN: 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 somatosensory zones of the posterior vermis of the rat cerebellum. We describe here the topographical organization of the projections from the pontine gray (PG), inferior olivary (IO), and medulla (MED) to the posterior vermal regions of lobules V-VI and their relation to the descending cortico and tectal projections to the brain stem. The midline cerebellar zone corresponding to the pontine reticular formation (PG), but a substantial input from the medial accessory olive (MAO). Lateral reticular nuclei (LRN) and caudal principal olive (PO). Lobules V and VIIa receive projections from the lateral reticular nuclei PO, whereas lobules VIIb, VIII and IX receive primarily from paramedian and lateral zones in the caudal PG. With respect to olivocerebellar projections, the posterior vermal lobules VIIa-VIIb receive primary projections from the medial PG, whereas lobules V, VIIa and b receive from the lateral MAO. All the midvermal lobules studied receive a projection from the caudal PG, with the exception of lobule VIIb, which receives solely from the MAO. In addition the studied lobules receive from paramedian reticular nuclei and/or Ia/IIa.

We conclude that pontine projections to the lobules VIIa and VIIb originate from areas receiving descending input from the visual (VC) and auditory cortices (AC). Pontine areas project to lobules VIIa and VIIb, whereas raphe projections are in accordance with the known descending systems to this structure, but these are not unique but rather the result of the formation of the projections from the visual (VC) and auditory (AC) cortices. Pontine areas project to the cerebellar cortex of the rat in a topographical manner.

PROJECTIONS BETWEEN TACTILE AREAS IN CEREBRAL (SI) AND CEREBELLAR (GC) CORTEX. Donald J. Woodward (SPCN: J. Kirkpatrick). Dept. of Physiology, Univ. TX Health Sci. Ctr., Dallas, Texas 75235.

We conclude that pontine projections to the lobules VIIa-VIIb originate from areas receiving descending input from the visual (VC) and auditory cortices (AC). Pontine areas project to the cerebellar cortex of the rat in a topographical manner.

CEREBRO-CEREBELLAR MICROCIRCUITS: MICROMAPPING THE FINE-GRAINED HIGHLY DISCRETE SET OF AFFERENTS. These can be expected to be physiological evidence for visual and auditory input to posterior vermis of the rat cerebellum. The midline cerebellar zone corresponding to the pontine reticular formation (PG), but a substantial input from the medial accessory olive (MAO). Lateral reticular nuclei (LRN) and caudal principal olive (PO). Lobules V and VIIa receive projections from the lateral reticular nuclei PO, whereas lobules VIIb, VIII and IX receive primarily from paramedian and lateral zones in the caudal PG. With respect to olivocerebellar projections, the posterior vermal lobules VIIa-VIIb receive primary projections from the medial PG, whereas lobules V, VIIa and b receive from the lateral MAO. All the midvermal lobules studied receive a projection from the caudal PG, with the exception of lobule VIIb, which receives solely from the MAO. In addition the studied lobules receive from paramedian reticular nuclei and/or Ia/IIa.

We conclude that pontine projections to the lobules VIIa and VIIb originate from areas receiving descending input from the visual (VC) and auditory cortices (AC). Pontine areas project to the cerebellar cortex of the rat in a topographical manner.

CEREBELLM TO CEREBELLUM: MICROMAPPING THE FINE-GRAINED HIGHLY DISCRETE SET OF AFFERENTS. These can be expected to be physiological evidence for visual and auditory input to posterior vermis of the rat cerebellum. The midline cerebellar zone corresponding to the pontine reticular formation (PG), but a substantial input from the medial accessory olive (MAO). Lateral reticular nuclei (LRN) and caudal principal olive (PO). Lobules V and VIIa receive projections from the lateral reticular nuclei PO, whereas lobules VIIb, VIII and IX receive primarily from paramedian and lateral zones in the caudal PG. With respect to olivocerebellar projections, the posterior vermal lobules VIIa-VIIb receive primary projections from the medial PG, whereas lobules V, VIIa and b receive from the lateral MAO. All the midvermal lobules studied receive a projection from the caudal PG, with the exception of lobule VIIb, which receives solely from the MAO. In addition the studied lobules receive from paramedian reticular nuclei and/or Ia/IIa.

We conclude that pontine projections to the lobules VIIa and VIIb originate from areas receiving descending input from the visual (VC) and auditory cortices (AC). Pontine areas project to the cerebellar cortex of the rat in a topographical manner.


Recently small neurons in the dentate nucleus of the rat have been shown to accumulate extracellularly injected tritiated gamma aminobutyric acid (3H-GABA, Cerebrol and Cerebellum). These results obtained following multiple injections of horseradish peroxidase in the cat brainstem (McCrea, et al., 1978, J. Comp. Neurol.). Indicated that all descending fibers to the dentate nucleus project to extracerebellar structures. Based on these findings, it may be inferred that neurons which accumulate 3H-GABA in the dentate nucleus in f.e., the visual cortex and auditory cortex (AC). Pontine areas project to the cerebellar cortex of the rat in a topographical manner.


Experiments were performed in unanesthetized decerebrate cats and were aimed at determining the organization of the 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-reticulo-spinal 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 dentato-rubrospinal projection could be eliminated by lesions in the lateral brachial 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 the stimulus current to the injected nucleus was ruled out by showing that the response was unaffected by electrolytic lesions in this structure adjacent to the injected nucleus. The segmental effect of these dentato-reticulo-spinal and dentato-rubrospinal projections was found to produce marked changes on interneurons and neurons as well as on reflexes mediated by segmental cutaneous fibers. 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 effects the excitability of neurons and reflexes mediated by cutaneous afferent fibers. This was supported by Grant# NS 09447.
177 IMPUT TO THE PARAFOCALUS: AN ELECTROPHYSIOLOGICAL STUDY. Richard A. Burns and Donald J. Woodward. Dept. Cell Bio., Inst. for Health Sci., Dallas, Texas. Previously we presented anatomic evidence suggesting that the paraflocculus is a cerebellar target zone for visual cortical and tectal input. This report brings forward the general aim of determining the electrophysiological properties of the visual sensitive Purkinje cells within the paraflocculus of the rat cerebellum. It is determined by a presentation 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 holothene 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 concententic electrodes, respectively (0.1 ms, 0.1-0.6 mA, 1-10 Hz).

Of 49 identified paraflocculus cells, the paraflocculus, PSTHs of 49 cells (8%) showed evidence of a mixed excitatory-inhibitory mossy fiber input (42 cells, 86%), or a pure inhibitory (5, 10%) or excitatory (4, 8%) input following cortical stimulation. Following tectal stimulation, 35 cells (80%) responded to the mixed excitatory-inhibitory mossy fiber input (24 cells, 59%), or a pure inhibitory (7, 14%) 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 ± 1.1 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 2.2 ± 1.7 msec and 7.2 ± 2.7 msec following cortical and tectal stimulation, respectively.

Single unit recordings of an additional 7 paraflocculus Purkinje cells in anesthetized, immobilized rats showed evidence, through PSTH analysis, for mixed excitatory-inhibitory mossy and climbing fiber input following visual field stimulation. The 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 0.3-18 m/sec.

In addition, off-on 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 the work supporting a strong visual input to the paraflocculus, and also indicate that a significant input from visual cortex and superior colliculus emerges upon paraflocculus. Our hypothesis is that the paraflocculus may serve as a strong link in visual sensori-motor integration.

178 ISOLATION OF OLIGOGLIOEDROCYTES FROM MOUSE CEREBELLUM USING MAGNETIC MICROSPHERES. Graham L. Campbell, Oded Abramsky,* and Donald H. Silberberg. Franklin Inst. Res. Labs. and Dept. of Physiology, Temple University Med. School, Phila., Pa. 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 bindign of ligands to cell surface moieties provides an alternative methodology. The development of magnetic microspheres to which ligands may be covalently attached and effective functional interrelationship between the vestibular and fastigial nuclear complex.

179 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 spinocerebellar pathways after similar neonatal lesions. Under hypothermic anesthesia, hemicerebellectomy lesions were compared to the excitability changes evoked in Purkinje cells by a natural stimulus. The pairs and triplets of Purkinje cells were identified by the presence of spontaneous climbing fiber responses. The natural stimulus consisted of stretch of the ipsilateral gastrocnemius-soleus muscle, with or without a plate in contact with the plantar aspect of the foot. The excitability changes evoked by the stimulus were determined by using 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 natural stimulus, 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 using PST histograms. The results suggested that there are two types of integrative processes occurring in the cerebellar cortex which may be relatively independent.
Topical DPH onto the motor cortex attenuated the cortical and thalamic epileptogenesis. Purkinje activities were suppressed during the tonic phase of thalamocortical bursts. Topical DPH prolonged Purkinje unit discharges with coincident abolition of thalamocortical firing; 2) following severance of the climbing fibers, Purkinje focal negativities occurred later; 3) following severance of the ipsilateral brachium conjunctivum, the spinal course of this projection suggests that a projection other than the DSCT originates from this nucleus. These experiments also defined the region of the nucleus dorsalis that is bilateral 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, 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. After a bilateral injection of the anterior cerebellar lobe following a lesion of the dorsal funiculus at T-9, labelled neurons in the contralateral nucleus dorsalis resulted from uptake both by ipsilateral DSCT terminals as well as by the terminals of bilateral projections coursing through the superior cerebellar peduncles. The nucleus dorsalis located ipsilaterally below the lesion contained only HRP positive neurons retrogradely labelled through the contralateral projection. The results were consistent with the hypothesis that spinocerebellar projections other than the DSCT originate in the nucleus dorsalis and project bilaterally to the superior cerebellar peduncles. This research was supported by NIH Grant # NS 05947. Dr. Tolbert was supported by NIH Fellowship # NS 05957.

Topographical projections of the deep cerebellar nuclei to the inferior olivary nuclei have been described in the monkey, cat, and opossum. Faul and Carman (1976) observed cerebellar-olivary fibers in the descending limb of the brachium conjunctivum after ablation of the inferior cerebellar peduncle. Chan-Palay (1977) has shown autoradiographically that the rat dentate nucleus projected primarily to the contralateral principal nucleus. At the level of the olivary complex 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 cerebellar 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 was observed. Preliminary results from microinjections of 3H-leucine into parts of the individual nuclei revealed that a much more complex topographical projection exists. The central part of the middle third of the 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). Injections to the inferior olive were observed. Preliminary results from microinjections of 3H-leucine into parts of the individual nuclei revealed that a much more complex topographical projection 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 half 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.)


Spreading depression (SD) and anoxia are two related phenomena. In the teleost fish cerebellum large decreases in [Na\(^+\)]\text{O} and [Cl\(^-\)]\text{O} 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. During anoxia, with normal Ringer superfusion, both [Na\(^+\)]\text{O} and [Cl\(^-\)]\text{O} fell to levels similar to those seen at the peak of SD, but recovery took place during the anoxic period. [K\(^+\)]\text{O} and [Ca\(^{2+}\)]\text{O} changes during both SD and anoxia resembled those reported previously (Nicholson et al, Proc. Natl. Acad. Sci. USA, 76: 1287, 1977).

The falls in [Na\(^+\)]\text{O} and [Cl\(^-\)]\text{O} during SD and anoxia in the rat cerebellum are similar, both in magnitude and proportion of normal baseline values, to those seen during SD in the monkey and in the opossum (Kraig and Nicholson, Neuroscience, in press). These results show that [Na\(^+\)]\text{O} and [Cl\(^-\)]\text{O} fall to relatively invariant levels during SD and anoxic depression 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.)


Albino rats were placed on a liquid diet (Ensure) containing either 9% or 5% ethanol during the second to twentieth day of pregnancy. Each group of animals was matched with a control group. 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; cerebellar and cerebral cortices and midbrain and cerebellum were measured. The results showed no significant difference in cerebellar development from the time of sacrifice to the last day postnatum. The weights of cerebellar and cerebral cortices were significantly higher in animals maintained in a nutrient free environment. 

The data indicate that the exposure of rats during embryonic and fetal life to alcohol, affects both cerebellar development and survival. Alcohol may interfere cerebellar development either directly or indirectly; in the latter case hypothryoidism may be one cause.
of the intermediate area of the cerebellar cortex has been con­ sidered to regulate the details of on-going movements in contrast to the lateral area which is involved with preprogramming a movement (Allan & Taskbara, 1974). Previous work (Thach, 1968,1970) showed that intact Purkinje zones Purkinje cells firing patterns during discrete phases of a stereotyped wrist move­ ment. 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 fore­ limb 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 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 fore­ limb response. Increases (54%) and decreases (26%) and biphasic (20%) responses were observed. The modulation of the firing pattern for an individual cell is displayed in Fig. 3B of 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 fore­ limb 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 oscilloscope mounted in front of the animal. A study of Purkinje cells (30%) related to the forelimb re­ sponse also responded to a tone which initiated each trial. This response was used as an stimulus to test the effect of a brief duration (<50 msec) that occurred independently of the fore­ limb response. No cells were found that responded to the presenta­ tion of the target light.

These results provide evidence that Purkinje cells in the inter­ mediate cerebellar cortex can regulate and reflect details of spe­ cific motor responses. The short latency response to auditory in­ put 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 FNS 10488 and McLaughlin Fdn. Fellowship to E.H.)
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 motoneuronal cord and spinal cord. The stereotyped postural responses elicited by stimulation of these structures reveals that local, spatially distributed parallel organization, being characteristic for the brain in general and for the cerebellum in particular, has allowed 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, 1980) will be demonstrated: in the cell level the theory identifies the function of individual Purkinje cells as taking different order derivatives of the parallel fiber input. The theoretical cell 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 NIMH).

TOPOGRAPHY OF DESCENDING PROJECTIONS TO THE INFERIOR OLIVIC 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 using the retrograde transport of horseradish peroxidase (HRP) revealed the sources of descending projections to the inferior olive from the upper brainstem. The cell of origin is centered in the rostro-medial area of the interstitial nucleus of Cajal (INC) and the nucleus parafascicularis and subparafascicularis, nucleus of Darkschewitsch (ND), interstitial nucleus of Cajal (INC) and the medial portion of the caudal third of the dorsal accessory olive. These cells were observed in nine adult monkeys (Cebus apella) under light thionental anesthesia. The anterograde transport of 3H-L-leucine (0.3-0.4 µl, 5-10 µCi) into the parafascicular region bilaterally 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.
CEREBELLUM

197


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 ventralanterior nucleus of the thalamus (VL) which are distributed in a gross topographical organization. Retrograde labeling with horseradish peroxidase (HRP) elicits a similar gross organization of the distribution of VL thalamic nuclei. Caudal parts of the fastigial nucleus terminate in ventromedial parts of VL and overlap with terminals from dentate nuclei in dorsal and rostral 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 precentral 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, with labeled cells found from HRP injections in hidden parts of the precentral gyrus. Projections from the interpositus posterior terminated in fewer, more closely spaced patches in mid-ventral VL. This area contained the locus ceruleus, substantia nigra, periaqueductal gray, and parts of the thalamus (VL) which are distributed in a gross topographical organization. Projections from these areas to the deep cerebellar nuclei terminate in patches in the ventrolateral nucleus (VL). These fibers were traced to the external cuneate nucleus and the subnuclei X and Z of the vestibular complex. Fiber degeneration was observed within the fasciculus and the granule cell layer of the cerebellum. The purpose of this investigation for a reciprocal innervation between these 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).

198


Previous work in our laboratory revealed that discrete stimulation (1-3 shocks, 50-500 µA, 0.1-0.2 m/sec) of raphe nuclei (central, dorsal, and intermediate) elicited 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. Considerable variation in response isocline, locus coeruleus (LC) stimulation, 50-250 µA, 0.1-0.2 m/sec) produced excitation with subsequent inhibition in the cerebellum. Furthermore, conditioning stimuli to either LC or VS-300 msec pre-stimuli did not alter the other response, though suppression of characteristic cerebellar responses. C-T paradigm studies suggested at least 2 possibilities: that the interaction of LC occur between the two nuclei or that the two nuclei in a different region is inhibiting the firing pattern of the other or direct interaction on cortex and fastigial nuclei. Since LC and LC terminate in the nigra, midbrain caudal, and granular layer of the cerebellum was electrophysiologically studied the first premise, an interplay of 5HT and NE neurotransmitter systems within the brain stem region. Anatomical findings have revealed that the raphe nuclei, 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 nuclei, LC, and VS were initially stereotaxically identified and verified by presence of characteristic pressor responses upon stimulation of these areas with brief impulses (10-15 msec). However, if stimulated at a precocceal level, the VC stimulation (1 shock, 50-500 µA, -pulse, 0.1-0.2 m/sec) evoked transynaptic spike activity within the VC. The 5HT and NE neurotransmitter systems identified by fixed latency and ability to follow up to 100-300 cycles/sec were recorded in LC following VC activation. All spikes identified by fixed latency and ability to follow up to 100-300 cycles/sec were recorded in LC following VC activation. All spikes identified by fixed latency and ability to follow up to 100-300 cycles/sec were recorded in LC following VC activation. The coefficient of the VC observed in the cerebellar cortex was calculated for both areas. In addition, LC and VS stimulation inhibited the firing pattern of spontaneously firing cells of the other area for 3-40 msec for the LC stimulation, in cats anesthetized with α choralose or decerebrated. (Supported by the Canadian Medical Research Council PG-1)

199

SPIKOBULAR AND SPINOCEREBELLAR PROJECTIONS IN THE RAT. Rand E. Swenson* and Anthony J. Castro. (SPON: Charles L. Webber, Dept. Anatomy, College of Medicine, Howard University, Maywood, Ill., 60153).

The normal distribution of spinocerebellar projections was determined in adult Long-Evans, black-headed rats using the Pindi-Heimer staining technique. Various lesions of the spinal cord were placed at l4 different spinal levels. Cells were sacrificed 1-7 days to 10 days postoperatively, and the brainstem and cerebellum were examined for axonal and preterminal degeneration. The major tract degeneration was found in the ventral and lateral funiculi of the ventrolateral portions of the brainstem. The ventrolateral tract gave rise to preterminal degeneration in the lateral part of the deep gray matter of the cerebellum. The subnucleus X of the medullary accessory olive, the lateral reticular nucleus (particularly its rostral portion), and the nuclear reticularis ventralis, gigantocellularis, and pontis capsularis. Degenerating fibers could be traced diverging from this bundle into the restiform body from which they were primarily distributed to the cerebellum with small innervation of the lateral vestibular nucleus and the subnucleus X of the vestibular complex. At spinal levels immediately caudal to the cervical nucleus of Y 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 ventrally into the thalamus. Caudal parts of the fastigial nucleus 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 the granule cell layer of the cerebellum. These fibers were traced to the external cuneate nucleus and the subnuclei X and Z of the vestibular complex. Fiber degeneration was observed within the fasciculus and the granule cell layer of the cerebellum. 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 showed degenerating cells included the locus cereus, substantia nigra, periaqueductal gray, superior olive and the nucleus commissuralis. Differences results from lesions will not be discussed. (Supported by NIH Grant NS 13230).

200


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 movement correction generates a command to terminate this movement correction on the basis of predictive information produced in the motor cortex, in addition to the primary motor cortex the basal ganglia. These observations suggest that the movement 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)

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 NIMH)
CEREBRAL CORTEX
Responsiveness of S1 cortical cells to receptive field thalamic and non-thalamic subcortical projections to cat's frontal cortex and of neurons projecting to the remaining prefrontal cortex, although varying somewhat in their topography.

The thalamic and limbic systems are differentially involved in short-term memory. In addition, they demonstrate that recovery of function from frontal damage is dependent on experience.

An aim of later experiments was to test the modulation of natural sensory input during a variety of motor behaviors. About 60% of cells in the forepaw area exhibited receptive fields 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.)
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 (X-rays) causes highly reproducible pattern of malformations in the developing nervous system. The degree of malformation is the result of a balance between the maturity of the neural tissue and the capacity for repair. Highlights of some experiments with 150R were: (1) with the 14th day embryos, there was a caudal band that appeared to be almost normal, but still sent axons to the cord. Possibly some CS neurons was abnormal. In 12th day rats 7 or 10 days old, the normal infant gap CS neurons were present. (2) The 14th day rats, despite disorder of early cell migrations, CS neurons developed a recognizable layer V corresponding to the caudal band and gang, but not the rostral band. Some CS neurons had bifurcated apical dendrites, the polarity of others was askew. In 15th day rats, this polarity was normal, and mature rats. In normal nature rats, CS neurons formed a caudal 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 into adult life, a kind of plasticity. Seventeenth day rats developed CS neurons in a recognizable layer V corresponding to the caudal band and gang, 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 disorganized in the rostral bands and the gap. CS neurons were mislaid in the ectopia, but still sent axons to the cord. Possibly the presence of gap neuron projections was caused by too few CS neurons being generated. In mature 18th day rats, CS neurons formed the two bands and gap nearly normally, but polarity of some CS neurons was abnormal. In 12th day rats 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)


Does neocortex in adults undergo substantial synap- tic reorganization after deafferentation as has been demonstrated in areas such as the septal nucleus or dentate gyrus? We have chosen layer I of neocortex to study this problem because layer I consists of a cellular nuclear group with identifiable band (layer I is narrow enough to allow sampling for quantitative electron microscopy and 3)afferent axons from the diencephalon terminate mainly in this layer while commissural afferents occupy the complementary inner portion of layer I. We examined layer I at various times after cutting the commissural fibers. Microdensito metric analysis of layer I was performed on normal adult opossums (D. virginianus) and the animals were allowed to survive 6, 30 or 60 days. In a second group of focal fibers from the preeebral band, it was possible source of new synapses in layer I, thalame- ncy was performed on some of the animals on survival days 30 or 60. An additional 6-day survival was allow- ed to produce the electron dense phase of anterograde degeneration. The aldehyde fixed brain was chopped in- to 50-µm slabs and every third slab was saved for fro- ze section histology (Nissl and Fink-Heimer stains). The remaining slabs were processed for electron micros- copy. We chose layer I cortex for our sample area since it receives a uniformly dense commissural projection deep to the input from the dor- sophrenal. Quantitative EM on layer I follows the procedures of Ebner and Colonlier (JCN, 179:261). Two results of these procedures are striking. First, the number of vesicle-containing profiles per unit volume 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 section 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 the ventral thalamicectom- aty at the end of 30 or 60 day survival periods. This degeneration field is unlike any seen in normal "animal" ma- terial after thalamecemy alone and lasts 6 days post oper. Our results suggest that at least layer I in adult mammalian neocortex can undergo synaptic reorganiza- tion and that thalamic fibers contribute to this pro- cess. (Supported by NIH grant NS-13031 and NS 05561.)

MULTIPLE UNIT RECORDING IN AUDITORY CORTEX OF CATS DURING DEAFENING. STEPHEN EVANS and GEORGE GREENE.

Previous attempts to describe functional connectivity with- in the auditory cortex have met with only limited success due to technical and, possibly, physiological problems. In physio- logical preparations involving paralyzed animals where adequate control of stimulation and recording conditions is possible, the physiological state of the animal is usually unsatisfactory. Since it is likely that the behavioral state of the animal is related to the degree of "articulation" 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. Acoust. Soc. Amer., 47(3): 1283-1287, 1970) 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., G. J. 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 sensory 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, 500-µ, thin walled). Individual bundles are connected to a differential amplifier to attenuate correlated activity (noise). Computers are used to control the behavior. Some data acquisition and analysis of spike trains, preliminary data are discussed.

Supported by NSO5606 and CN00994.

DIFFERENTIAL DEPRESSANT EFFECTS OF N. RAPHE DORSALIS STIMULATION ON ELECTROCYTOGICAL ACTIVITY IN CATS. J. García Ramos. Physiol. Lab., Escuela Médico Militar, México D. C. H. MEXICO.

It has been shown that N. Raphe dorsal stimulation depresses the basal electrocortical activity, evoked potentials and strych- nine 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 occurs only 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 induced by low- voltage, high-frequency waves. The observations were made on unanesthetized cat. The obtained results cortical activity was obtained by constant stimulation of anfferent somatic nerve at a rate of 50 to 100 per sec, or by sound stimulation with white noise, between 50 and 80. The cortical areas explored were the somatosensory auditory, and an associative one such as the suprasylvian gyrus. The obtained results were similar in all of them, thus supporting the idea that the labeled serotonin acts in a diffuse manner as a neuro- humoral substance. It is suggested that a similar mechanism might be involved in the process of attention in which low relevant in- formation would be blocked, leading dendritic membranes in a con- dition under which high-value information could find priority for its processing by the brain.
DEGENERATION ARGYROPHILIA FOLLOWING LONG-STANDING DAMAGE TO THE
maps of this region. The dendritic trumpet analyses of pyramidal
random vertical distribution.
contours observed by others in electrophysiologically obtained
cell basal dendrites also show that they have a distinctly non-
prefor orientation of the dendritic sticks in an approximately
the experimental neuroanatomist interested in the human brain. We
fellowship to M.R.G.
the peripheral nervous system where degeneration is cleared within
a few weeks, or the CNS of experimental animals where degeneration
after the initial response to cell death. This contrasts with
minalis The second case had cerebrovascular damage which occur­
afeer weeks, or the CNS of experimental animals where degeneration
its centroid, its moments about the dendrite origin, and the dis­
dividual approximating chord segments of about 25µm length. The
degenerating fibers and terminal arborizations were demonstrated
in the human brain, we have previously reported a case in which
in the human brain, we have previously reported a case in which
Lausanne, Switzerland.

Electro生理ologicah observation suggests that in the
corticostriate fibers, and the stria ter­
meticulosa The second case had cerebrovascular damage which occur­
with some medial and basal areas of the cerebral cortex: namely
with the cingulate gyrus (areas LA and LC), medial frontal cortex
(F3), the orbital frontal, medial frontal, temporal polar and parahippoca-
portions related to specific limbic or paralimbic sectors of
the cerebral cortex: namely the temporal pole, inferior parietal lobe, and prefrontal cortex. Our recent
observations suggest that in addition to these well known rela-
relationships, the medial pulvinar has an abundance of connections
with some medai and basal areas of the cerebral cortex: namely
more heavily stained neurons in 100µm and 300µm thick sections.
NIH grants #NS 09518 and NS 05612. Price, JCN 178:711). Conduction velocities (Vc) (calculated as


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)
provide strong evidence for a recording position near the soma.
Lesions were made at the recording sites for 13 units. The
were demonstrated for 1 unit. Evaluation of Tc indicates that axon-
directly caudal to the dprocessor of medial dorsal and ventral (MD and VTA) injections of HRP or THM in more-central
areas were contralaterally injected. Since the medial pulvinar
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
labeled, in some cases more heavily.

primary and related systems (100, 112). Conduction velocities (Vc) (calculated as


APs) that afferent and efferent activity can be transmitted from the thalamic nuclei to the cerebral cortex and vice versa. The
medial pulvinar is one of the largest nuclei in the thalamus, and it
projects to many areas of the cerebral cortex, including the primary and secondary auditory cortices. The medial pulvinar receives input from the lateral geniculate nucleus and projects to the auditory cortex. The medial pulvinar is involved in the processing of auditory information, including the detection of sound location and the discrimination of speech sounds. The medial pulvinar has also been implicated in the processing of emotional and attentional information. The medial pulvinar is a critical target for basal ganglia lesions, such as those caused by Huntington's disease, which can result in dysfunction of the medial pulvinar and other thalamic nuclei. This can lead to symptoms such as dystonia and chorea, which are characteristic of Huntington's disease. The medial pulvinar is also involved in the processing of sensory information, including the detection of pain and temperature. The medial pulvinar is connected to the primary somatosensory cortex, which is involved in the perception of touch and pressure, and it receives input from the thalamus and the spinal cord. The medial pulvinar is also involved in the processing of visual information, including the detection of visual patterns and the recognition of objects. The medial pulvinar is connected to the primary visual cortex, which is involved in the perception of visual patterns and the recognition of objects. The medial pulvinar is also involved in the processing of motor information, including the planning and execution of movements. The medial pulvinar is connected to the primary motor cortex, which is involved in the planning and execution of movements. The medial pulvinar is also involved in the processing of emotional information, including the processing of pain and the regulation of emotional responses. The medial pulvinar is connected to the primary limbic cortex, which is involved in the processing of emotional information. The medial pulvinar is also involved in the processing of cognitive information, including the processing of language and the regulation of attention. The medial pulvinar is connected to the primary language cortex, which is involved in the processing of language and the regulation of attention. The medial pulvinar is also involved in the processing of motor information, including the planning and execution of movements. The medial pulvinar is connected to the primary motor cortex, which is involved in the planning and execution of movements.
214 ANOMALOUS SYNAPTIC CHEMISTRY IN ADULT RAT NEOCORTEX FOLLOWING PRENATAL TREATMENT WITH METHYLXAMOXYMETHANOL. 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 16 of gestation (DG) with 20 mg/kg of methyloxamoxymethanol (MAM), a potent nicotine acid alkylating agent, was examined with regard to the effects on presynaptic markers and postsynaptic receptors on component neurons. Treated rats gained weight normally but were microencephalic. The MAM treatment reduced the weight of the forebrain by 33% and of the lateral cortex by 67% and the weight of the hindbrain was increased. Neocortical cytoarchitecture was strikingly altered, and cortical layers II and III were nearly absent.

As a consequence of the prenatal treatment the GABAergic neurons — glutamate decarboxylase, endogenous GABA and [3H]GABA uptake by P fractions — were minimally altered in the MAM-treated cortex; however, the total amount of the markers in the cortex were reduced by 60-70% (p < 0.01). The specific binding of [3H]GABA to cortical membranes was similarly affected. In contrast, the concentrations of the presynaptic markers for the cholinergic neurons — glutamic acid decarboxylase, 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 [3H]quinuclidinyl benzilate to muscarinic receptors was reduced by 40% (p < 0.01). The specific binding of [3H]glutamate uptake by P2 fractions — were minimally altered in the MAM-lesioned cortex whereas more posterior lesions did not. Lesions restricted to individual subfields of the frontal cortex (medial, orbitofrontal, motor) produced similar effects. The sum of the small lesions equaled 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 result of the improved spared cortical function 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 IT might produce such a deficit. This investigation addresses the question of whether temporal pattern dependence 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-soft-soft to loud-soft-soft 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 a 24 to 48 hour survival period the animals were retrained. The protocol and results were similar. The granular capping of the background which is interpreted as being located in the terminal branching of axons after anterograde transport by cells is in area 19, or along recurrent collateral of the retrogradely labelled cells. Both labelled cells and terminal arborizations appear in clusters or columns which are generally strongly superimposed. In area 17, both pyramidal and stellate cells are labelled. The pyramids are especially numerous in layers II and III but are also prominent in IV, V, VI and VII. The stellate cells are concentrated in layer IV. Fine anterograde labelling is practically absent from IVb, and densest in IVA and the lower part of II. A prominent tangential staining is in area 19, labelled pyramidal cells are seen in layers I, II, III, IV and VI but are much fewer in number. Fine anterograde labelling is virtually absent from layer IVv. This is most intense in IV, and only a few scattered granules are present in I. In the contralateral area 18, labelled cells are numerous but restricted to layers II and III. Discrete anterograde grains are nearly absent in IV, and only a few scattered granules are present in I. In the corticopontine fibers, the maximum concentration is in the lower 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 centrifugal pattern of the clusters decreases by several tens of per cent. Several pyramids more peripherally. The periodicity suggests that they relate to olfactory dominance columns. Supported by MRC.
A NORADRENERGIC PROJECTION TO THE BARREL HOLLONS OF MOUSE SOMATOSENSORY CORTEX.

Hart C.W. Lidow, Frank L. Rice, and Mark E. Molliver. Departments of Cell Biology/Anatomy and Neurology, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205. U.S.A.

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 was 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 fluorescence 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. We propose that 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.

It was the intent of this study to focus on two general categories of neurons have been described in the primary visual cortex (area 17) of the cat, those receiving input from the thalamus and those receiving input from more than one thalamic nucleus (Brain Res. 96:51, 1975). We wished to extend on these findings in the cat by comparing cortical layers of termination and the ultrastructure of monoaminergic endings made by these thalamic regions. For light microscopy triitated amino acids were iontophoretically or mechanically deposited in the appropriate thalamic nuclei. 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 nucleus (PN) and the pulvinar-lateral posterior nucleus (LP), the latter contained glial cell bodies but rarely a neuron. Its main compartment 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 axons population was the one which degenerated. In these latter cases it was the small boutons in deeper layers which showed evidence of degeneration.

CEREBRAL CORTEX

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


The massive input to the basilar pontine nuclei (BNP) 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-Imp 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 subtypes, those with relatively spine-free dendrites (aspiney) and those exhibiting numerous spines along with a variety of other somal or dendritic protrusions (spinsey). It was the intent of this study to focus on the spiny 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), completely contacted small dendritic or spiny profiles and exhibited all the typical features of electron dense degeneration. The other group was larger on the average (to 2.2 µm), contacting both small and intermediate sized dendrites and exhibited an initial filament or filamentous reaction prior to becoming electron dense. When lesions were confined to sensorimotor cortex, primarily small 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 axons project to the pontine nuclei. Furthermore, when animals were processed according to combined Golgi-Imp procedures, isolated spiny relay cells were contacted by dark boutons suggesting a convergence of these two cortical afferent systems.

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

OXYGEN DISAPPEARANCE RATES IN THE GERbil CORTEX UNDER HYPERBARIA.


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 arterial PO2 and below that of hemoglobin saturation but at higher values (PO2 > 130) became PO2 independent. These results are in agreement with those previous reports "made with labeled oxygen" and the basis for our interpretation is the oxygen consumption presented by Renau (JAICE 15: 916, 1969) i.e., at lower PO2 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.

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

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

John E. Miller, Mark Huschman and Louis A. Benvenuto. Departments of Anatomy, College of Medicine, University of Illinois Medical Center, Chicago, Illinois 60612.

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 extend on these findings in the cat by comparing cortical layers of termination and the ultrastructure of monoaminergic endings made by these thalamic regions. For light microscopy triitated amino acids were iontophoretically or mechanically deposited in the dorsal lateral geniculate body (LGB) and the posterior thalami. 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 from more than one thalamic nucleus (Brain Res. 96:51,1975). 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 branched and thin cylinders. The majority are 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 thalami (which spared the LGB) the scattered, poorly myelinated axons 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)
DEGENERATION OF THE COERULEO-CORTICAL PROJECTION: ORGANIZATION AND IMMUNOHISTOCHEMICAL CHARACTERISTICS. J. E. Morrison, W. M. Muller & R. G. Grama* Dept. of Cell Biology & Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Immunofluorescent (IFL) staining using a homologous antiserum demonstrates a profound loss of the coeruleo-cortical projection. This antiserum is directed against rat dopamine-beta-hydroxylase (DBH) demonstrated an abundant noradrenergic (NA) innervation in all areas of neocortex. The distribution of NA axons is characterized by a geometric organization of cortical barrels. We have utilized a variety of lesions to analyze this projection and to demonstrate more definitively the specificity of the IFL staining. The morphological and functional integrity of the NA projection, therefore, is maintained and can be identified in the moribund and injured animal. In order to show that the lesion is a specific marker for NA neurons, it is necessary to demonstrate the absence of staining following lesions selective for complete denervation of the area under study. Hence, we have made a series of bilateral electrolytic lesions of the dorsal NA bundle in the midbrain and a series of bilateral microlesions followed by lesion isolation. The results suggest that the antiserum may be returned to control values, the biochemistry is unchanged, and the distribution of the IFL specific staining pattern is unchanged. The most frequently employed controls for IFL specific staining. The most frequently employed controls for IFL specific staining include staining with normal serum or with DBH IFL. The distribution of NA axons is characterized by a geometric organization of cortical barrels. We have utilized a variety of lesions to analyze this projection and to demonstrate more definitively the specificity of the IFL staining. The morphological and functional integrity of the NA projection, therefore, is maintained and can be identified in the moribund and injured animal. In order to show that the lesion is a specific marker for NA neurons, it is necessary to demonstrate the absence of staining following lesions selective for complete denervation of the area under study. Hence, we have made a series of bilateral electrolytic lesions of the dorsal NA bundle in the midbrain and a series of bilateral microlesions followed by lesion isolation. The results suggest that the antiserum may be returned to control values, the biochemistry is unchanged, and the distribution of the IFL specific staining pattern is unchanged. The most frequently employed controls for IFL specific staining include staining with normal serum or with DBH IFL.
MEMBRANE PROPERTIES OF IDENTIFIED HUMAN CORTICAL NEURONS. D.A. Prince, R.K.S. Wong and A.J. 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 and whole cell recording, has enabled us to begin to examine the membrane properties of identified human neocortical neurons. Eighteen high quality stable intracellular recordings were obtained from 11 cortical regions in 3 patients. In all cases removal of normal cortex was required to approach a remote lesion. Three neurons showed short duration and high amplitude 1 msec and a single neuron in area 10 showed a longer duration 2 msec with increasing depolarizing current. One of these cells was labelled with HRP and appeared to be a spiny stellate interneuron. Fifteen neurons produced long duration (<2 msec) rectification which was not present in the control slice of the same hemisphere. These neurons were labelled with HRP 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 (Ri) 39M (50-50 M); time constant 36.7 msec (25-55 msec). Fourteen of the 15 cells with >1 msec duration spikes showed sub-threshold nonlinear membrane properties which were both voltage and time dependent. During depolarizing current pulses there was an apparent increase in Vm which began about 20 milliseconds after the onset of depolarization. Such anomalous rectification has not been previously described in neocortex. Nine of 18 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 mV, with durations of 30-200 ms. The space-clamp properties of the current pulse were altered by the presence of anomalous rectification, depolarizing afterpotentials, and post activation hyperpolarizations suggest a possible role of calcium in membrane conductance regulation. Further studies of differences in properties of pyramidal type cells and interneurons are supported by NIH Grants NS56477, NS12151, NS11614, and DA01949.

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 sodium channel blocker desmethyliprimipam or by preoperative destruction of catecholaminergic neurons with 6-hydroxydopamine (Biol.Psychiat. 12:669,1977) and the catecholamine agonists NE and DA01949.

Following right middle cerebral arterial ligation (WM14) we compared sham operated control animals, those with 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 sodium channel blocker desmethyliprimipam or by preoperative destruction of catecholaminergic neurons with 6-hydroxydopamine (Biol.Psychiat. 12:669,1977) and the catecholamine agonists NE and DA01949.

The current experiments were carried out using cages which allowed free access to food, water and a running wheel. Following right middle cerebral arterial ligation (WM14) we compared sham operated control animals, those with 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 sodium channel blocker desmethyliprimipam or by preoperative destruction of catecholaminergic neurons with 6-hydroxydopamine (Biol.Psychiat. 12:669,1977) and the catecholamine agonists NE and DA01949.

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 sodium channel blocker desmethyliprimipam or by preoperative destruction of catecholaminergic neurons with 6-hydroxydopamine (Biol.Psychiat. 12:669,1977) and the catecholamine agonists NE and DA01949.

The current experiments were carried out using cages which allowed free access to food, water and a running wheel. Following right middle cerebral arterial ligation (WM14) we compared sham operated control animals, those with 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 sodium channel blocker desmethyliprimipam or by preoperative destruction of catecholaminergic neurons with 6-hydroxydopamine (Biol.Psychiat. 12:669,1977) and the catecholamine agonists NE and DA01949.

CORTICAL 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 0.5% 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 more anteriorly and lateral to the 5a region, 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 caudal to area 5b, receives prominent projections from LP and the rostral pulvinar (Pu). In addition, retrograde labelling indicates light projections from CL, VL, and Pom. The ventral anterior nucleus (VA) sends light projections to area 7 of the suprasylvian gyrus, but not the marginal gyrus.

Supported in part by NIH grant NS 14267.
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 key choices; whereas these 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 rewards for correct choices 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 firing 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 NS10244, EY01255, NS07057-02.)


Layer IV of Sm1 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 the 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 spindled dendrites whereas the dendrites of class II cells are smooth with beaded branches. 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 barrel. 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, FY01255, NS07057-02.)


Layer IV of Sm1 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 the 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 spindled dendrites whereas the dendrites of class II cells are smooth with beaded branches. 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 barrel. 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, FY01255, NS07057-02.)


Many studies have shown that the cingulate and subicular cortices project to the lateral dorsal nucleus (LD) of the thalamus. However, the cortical 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 II 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 tip 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 external 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.)

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 paralobe of the rhesus monkey, a distinct architectonic zone has been identified in the lower bank of the intraparietal sulcus. This area 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 (PO) 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 parietal belt, especially the lateral surface of the hemisphere. After injections in the posterior part of the cingulate cortex. The remainder passed dorsally where they entered the fasciculus cinguli and projected to the ipsilateral anterior cingulate cortex. 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, NIMH grant NS 09211 and V. A. Research Project #6901.
UNILATERAL ABLATION OF THE ENTIRE ECTOSYLVIAN REGION HAS BEEN SHOWN TO RESULT IN SPECIFIC CHANGES IN THE PATTERN OF VISUAL CHOICE IN DOUBLE SIMULTANEOUS BILATERAL STIMULATION (DSS). THIS REGION HAS BEEN SHOWN TO PROJECT DIRECTLY TO THE HOMOLATERAL RHINAL CORTEX AS WELL AS TO 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 PROBABILITY THAT PSYCHOPHYSICAL BEHAVIORAL REFLECTS HEMISPHERIC IMBALANCE WHICH CAN BE ALTERED TOWARD NORMAL BY A CONTRALATERAL LESION IN THE ADJACENT SUPRASYLVIAN CORTEX.

Eleven adult female cats were used. They were first trained to enter either of two adjacent, alternating mazes, a response that was learned reliably. Correct responses were rewarded by food. The subjects were trained to choose the illuminated maze when only one was lighted, and could select either compartment when both were lighted. The latter condition was DSS and presented randomly 20% of the time. Most animals demonstrated a hemineglect 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 restested.

When a subject with unilateral cerebral damage responded at preoperative levels to single stimuli and its behavior 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 the hemisecting of the visual cortex.

Within the temporal lobe, only lesions which included the posterior ectosylvian gyrus resulted in unilateral visual neglect.

Finally, if subsequent contralateral ectosylvian or suprasylvian cortex was ablated, the degree of neglect lessened and task behavior approached that of the original state. The functional mechanism thus appears to involve the brain as a brain-tobrain system stored to structural balance. This study then contributes to understanding of the role of the EPR cortex as an area involved in the regulation of visual behavior. Since it also receives projections from AM1 and the auditory thalamus, it may play a role in auditory-visual association.

PROJECTIONS FROM THE ANTERIOR ECTOSylvIAN GyrUS TO THE PERIRHINAL CORTEX. K. Yamaguchi and S. Horenstein. Saint Louis University, Saint Louis, Missouri 63104.

It has been shown that the anterior ectosylvian gyrus has direct projections 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 probability that psychophysical behavior reflects hemispheric imbalance which can be altered toward normal by a contralateral lesion in the adjacent suprasylvian cortex.

Eleven adult female cats were used. Correct responses were rewarded by food. The subjects were trained to choose the illuminated maze when only one was lighted, and could select either compartment when both were lighted. The latter condition was DSS and presented randomly 20% of the time. Most animals demonstrated a hemineglect of response preference, the other side being referred to as subordinate. After preoperative learning and testing, one or another portion 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 restested.

When a subject with unilateral cerebral damage responded at preoperative levels to single stimuli and its behavior 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 the hemisecting of the visual cortex.

Within the temporal lobe, only lesions which included the posterior ectosylvian gyrus resulted in unilateral visual neglect.

Finally, if subsequent contralateral ectosylvian or suprasylvian cortex was ablated, the degree of neglect lessened and task behavior approached that of the original state. The functional mechanism thus appears to involve the brain as a brain-to-brain system stored to structural balance. This study then contributes to understanding of the role of the EPR cortex as an area involved in the regulation of visual behavior. Since it also receives projections from AM1 and the auditory thalamus, it may play a role in auditory-visual association.

This report is a continuation of studies on the histopathological effects of high frequency electrical stimulation of the cerebral cortex using platinum and rhodium electrodes. The work comprises a part of a Neural Prosthesis Program whose ultimate aim is to develop techniques for replacing lost function in persons with varied neurological deficits.

In previous brain stimulation experiments neural damage was assessed with respect to the amount of charge and the parameters of current density per phase (QD/ph), charge density per phase (QD/ph) and current density per phase (J/ph) when varied in 16 separate combinations. Although neural damage was determined as a function of electrical and chemical parameters, the extent of damage was not predictable from the parameters. The extent of neural damage was defined as the extent of electrical and chemical stimulation that was necessary to produce damage in the neural tissue.

In this study the relationship of QD/ph to neural damage has been investigated using surface stimulation of the parietal cortex of cats. Light and electron microscopic studies were carried out on electrode sites of animals stimulated over a QD/ph range of 10 to 300 μC/cm²/ph for 36 hours (9 hr./day) stimulations. QD/ph variations were achieved by altering the electric current (1.1 to 3.6 mm dia.) or QD(0.1 to 3.0 μC/ph). The neural damage threshold was observed at a QD/ph of approximately 20. Seizure activity appeared at a QD/ph of 40 μC/cm²/ph, and there was an increased amount of seizure activity with QD/ph up to 100 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph). For example, severe generalized seizures were observed at an electrode site at a QD/ph of 10 μC/cm²/ph and QD(0.1 to 3.0 μC/ph).
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 licks 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 Prützel, Psychol. Bull., 1977, 85, 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

Intracellular recordings from taste receptor cells and nerve recordings from the lingual nerve indicate that those receptor sites which give rise to intracellular taste obey the same physiochemical laws as those at the apical surface of the taste cell. The response of the taste cell to intracellular stimulation is primarily determined by the 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 a non-oxygenated perfusate (Landis, Am J Physiol 83:528, 1928). By restricting percutaneous O, absorption and by using non-oxygenated perfuses, intracellular recordings to NaCl, LiCl, CaCl, Cl, CH3COOH, quinine-HCl, sucrose and saccharin were obtained. Tentative evidence suggests that the taste response profile of a receptor cell as determined by intracellular 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=6). It is suggested that muscular movement may help clear the extracellular fluid space in the tongue in a manner analogous 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. Stimulation of interaction with intravascular taste receptor sites is constrained by access to and clearance from the extracellular fluid space, when these parameters are manipulated the intracellular receptor sites seem to follow those equations developed by Beidler (J Gen Physiol 38:133,1954) to characterize the apical taste sites.

Supported by MINDS Grant 1NS07068-02.


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 trigeminal after subcutaneous injections of [3H]-proline in various parts of the trigeminal ganglia confirms earlier classical descriptions and further suggests that the trigeminal fibers which enter the ventrolateral portion of the nucleus of the trigeminal tract (NST) originate from cells in the ophthalmic segment of the ganglion.

Injections of the geniculate ganglion labels fibers of the VIIth nerve which both ascend and descend NST. Some ascending fibers distribute in a compact and circumscribed zone immediately dorsal to the spinal V nucleus as far rostrally as the commissural part of NST. Moreover, only X appears to have a significant ascending component of IX and X in the spinal V tract is present only when the superior ganglion of either nerve is involved by the 3H-proline deposit. Supported by NSF grant BNS 76-81408 and NIMH grant 15125.


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 laikward migration of sockeye salmon (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 ions is involved by the 3H-proline deposit. Supported by NSF grant BNS 76-81408 and NIMH grant 15125.

Olfactory and gustatory systems of the channel catfish, Ictalurus punctatus, are the highly sensitive sensory organs (Caprio, J. Natrue 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 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, eliminated the responses to the other L-amino acids except L-arginine at 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 contained primarily alanine sites, but also have binding sites for other amino acids.

Olfactory cross adaptation experiments have shown that any of the effective amino acids, except alanine used as adapting stimuli, depress to varying degrees but not abolish the responses to the other amino acids. Presently, there is no evidence of specific binding sites which are innervated by arginine-best taste neurons. Other taste cells innervated by alanine-best taste fibers were also cross adapted to other L-amino acids. (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.)


Three days of differential olfactory exposure before, but not after sexual maturation, influenced the odor preferences of Sprague-Dawley rats. Three days before testing, rats were placed in either cedar or pine shavings in isolated olfactory environments. On the fourth day, rats were given two odor preference tests in apparatus which allowed animals to smell but not touch or taste stimuli shavings.

Pine-housed neonates 11 days old and juveniles 30 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 odor.

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 adaptation 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 in adult odor exposures. Thus, the results may have been induced tolerance of juveniles for natural cedar odor, but not their preference for cedar nest odor. The data therefore imply changes in central rather than peripheral neural substrates.


The routes and terminations of projections to the main olfactory bulb (MOB) from the anterior olfactory nucleus (AON), hippocampal pulsumidial (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 bulbular limb of the anterior commissure (AC). These fibers were distributed 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 regions of the AON revealed bilateral projections to the MOB via the AC. The AON fibers passed ventromedially from the AC to enter the dorsal aspect of the MOB and dorsomedially to the factory bulb to enter the more anterior part of the MOB. Striking differences were seen in the termination patterns of the centrifugal projections to the subdivisions of the AON. Injections in the lateral or caudal peduncle involving primarily pars laterale, pars ventralis or pars posterior produced a heavy terminal pattern of granular autoradiographic grains in 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 the lateral or caudal peduncle involving primarily pars laterale, pars ventralis or pars posterior produced a heavy terminal pattern of granular autoradiographic grains in 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 the mediodorsal sector 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 MOB 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 ipsilateral projection to the pyriform cortex (LOT). The LOT component ventrally and dorsally to 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 fiber 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)


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:

<table>
<thead>
<tr>
<th>Region</th>
<th>Control side</th>
<th>Lesion side</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOT</td>
<td>4.70±0.5</td>
<td>4.81±0.7</td>
</tr>
<tr>
<td>Layer IA</td>
<td>13.10±0.8</td>
<td>13.74±1.4</td>
</tr>
<tr>
<td>Layer II</td>
<td>16.80±0.7</td>
<td>16.21±1.8</td>
</tr>
<tr>
<td>Layer III</td>
<td>16.80±0.7</td>
<td>16.41±3</td>
</tr>
<tr>
<td>Glutamate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOT</td>
<td>21.3±1</td>
<td>7±1</td>
</tr>
<tr>
<td>Layer IA</td>
<td>48±2</td>
<td>30±3</td>
</tr>
<tr>
<td>Layer II</td>
<td>66±2</td>
<td>62±2</td>
</tr>
<tr>
<td>Layer III</td>
<td>57±2</td>
<td>56±1</td>
</tr>
<tr>
<td>Aspartate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOT</td>
<td>8.40±0.6</td>
<td>1.80±0.3</td>
</tr>
<tr>
<td>Layer IA</td>
<td>12.21±0.0</td>
<td>7.30±0.3</td>
</tr>
<tr>
<td>Layer II</td>
<td>16.15±0.7</td>
<td>12.21±0.0</td>
</tr>
<tr>
<td>Layer III</td>
<td>14.3±0.1</td>
<td>12.8±0.7</td>
</tr>
</tbody>
</table>

Both glutamate and aspartate levels were significantly reduced in the LOT, which contains the axons projecting to the cortex from the olfactory bulb (a 10 decrease). In 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 BC45 and USPHS grants NS-08862 and NS-08000).
CHEMICAL SENSES


VIIth nerve was cut at the stylomastoid foramen. Dry flakes of GSP series may be due to axonal branching or to the presence of GG near the VIIth nerve. Labeled cells are scattered in the GG, with a few in the 30-40um range. CT incubation yields a mean of ±

248 Effect of Intranasal Irrigation of Nitric Inhibitors on Olfactory Behavior and Biochemistry in Mice. J. W. Harding and J. M. Wright* Washington State University, Pullman, WA 99164.

Mice were intranasally irrigated daily with 100 microliters of normal saline, hydroxyurea (10 μM), and ethidium bromide (10 μM), or weekly with hyperosmolar saline (920 mOsm/kg) and lampbrush silicic acid (247 mOsm/kg). The mice were pretrained to find buried food pellets (45 mg) or amyl acetate (5 μl of 1:40,000) in the different experimental arms. The mice were trained to find buried food pellets or sugar cubes. There was no change in the performance of the saline animals while the other groups exhibited a modest and temporary decline in olfactory capabilities. Following 21 days of treatment the experiment was terminated. The behavioral results were confirmed by in vitro measurement of H-thymidine incorporation showing a significant decrease in total incorporation by olfactory epithelium for all inhibitors tested. (Supported in part by NIH Grant NS 10389.)


Continuing neurophysiological study of central projections of the olfactory bulb (OB) in the pigeon has shown that direct OB efferents terminate in the contralateral parolfactory lobe (PLO), nucleus accumbens (Ac), and the preoptic area (POA), and the intrapreduncular nucleus (IPN). Our pilot electrophysiological work had provided confirmatory evidence of paleostratal connections. More extensive confirmatory data can be expected from the continuing behavioral studies in various central and peripheral sites. Response properties characteristic of monosynaptic stimulation were recorded in PFP, PA, and LPO. Unit activity of PA was enhanced by stimulation, whereas units in LPO generally showed depression, results consistent with our previous findings in the same structures ipsilaterally. Cell populations were studied in parallel with the previous work had shown to be consistently inhibited by ipsilateral ON stimulation, showed only excitation during contralateral ON stimulation. Peak firing rate, consistent with the peak latency of the evoked potential recorded through the same electrode, have been observed in these areas when the analysis was completed. High stimulation intensity and high stimulation frequency resulted to show that contralateral ON stimulation enhances firing rates of cells in ventral hyperstriatum and AHP. (Supported by USPHS grant NS 10389 and NIH-MDS 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 circumvalate and foliate papillae of the tongue. Differences in gustatory sensitivities among the tongue appears to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter. The differences in the gustatory sensitivities of the tongue appear to be ascribed to differences in axon diameter.
Ofactory input to the main olfactory bulb and vomeronasal organ input to the accessory olfactory bulb are essentially normal and aggressive behavior in male hamsters (Winans & Powers, Brain Res., 126: 325; Murphy, Brain Res., 113: 95). Similar neurons are aggregated in the rostral or caudal part of the corticomedial amygdala region) of the female. Males with damage confined to the caudal part of damage to the rostral part of the corticomedial nuclear group.

Animals mating behavior deficits in lesioned animals ranged showed some increase in latency to ejaculation, electrolytic lesions of the stria terminalis (n=7) or sham operations led to similar deficits.

This suggests that non-strial efferents to the mating behavior of male hamsters before and after bilateral electrolytic lesions of the corticomedial amygdala. This suggests that non-strial efferents from the olfactory bulb and vomeronasal organ input to the accessory olfactory bulb are essential for organizing taste buds on the rostral 15mm of tongue surface. An average S.D. of 35±15% of total fungiform taste buds degenerate when A is severed, but only 13% are located on the anterior 4 mm. An arc of taste buds cells to the anterior 4 mm with ipsilateral section of the entire chorda tympani nerve and these are innervated by axons which cross the midline, the mid-aural branch, C, innervates axons which cross the midline. A posterior branch is anterior to the intereminal eminence 12-20 mm from the tongue tip. Posterior branches D and E innervates 5±3% of fungiform taste buds lost to the olfactory bulb. Aggression 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 buds to cells as posterior branch to the intereminal eminence.

Since the major outflow of the corticomedial amygdala to the hypothalamus is by way of the stria terminalis, we also studied either the rostral or caudal part of the corticomedial amygdala. This suggests that non-strial efferents from the rostral corticomedial amygdala play a major role in the mating behavior of male hamsters.

**ODOR DISCRIMINATION AND MEMORY IN KORSACKOFF'S PSYCHOSIS.** Robert G. Nairn, Cynthia Capra*, William J. McBeth, and Tyrone Engle

Korsakoff's psychosis is characterized by severe memory impairment, associated with consistent patterns of diencephalic and medullary lesions. The hippocampus has been damaged to 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. 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 the majority of the cognitive tasks of Korsakoff patients with catatheolin agonist drugs which have improved performance on other memory tasks, had no effect on odor memory. These results suggest that the Korsakoff patient's impairment in their ability to discriminate between two odors, but not in their ability to detect odors. The failure of catatheolin 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.

**SUPPOPULATIONS OF FUNGIFORM TASTE BUDS IN THE RAT.** Edward H. Lubarsky* and Ingela J. Miller, Jr. Dept. of Anatomy, Bowman Gray Sch. of Med., Wake Forest Univ. Med. Center, Wake Forest, NC 27159

It has not been established whether taste buds function primarily as individuals or in groups, but lateral depression and dissection 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 which demonstrate electrophysiological changes in 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 rat animals. Electrolytic bilateral lesions or surgic­

**AN APPROACH TO CHEMICAL NERVE SECTION IN THE MOUSE OLFATORY PATHWAY.** F.L. Margolios and N. Grill.

The use of a neurotoxic agent to selectively destroy or block the formation of new neurons in the olfactory epithelium has become a new tool for the analysis of developmental and functional relationships in the nervous system. Intranasal irrigation with 0.17 M NaSO3 solution has previously been shown to have a longitudinal effect on the morph-ology and biochemistry of the olfactory bulb and mucosa, as well as on food-finding behavior (Brain Res. 100, 271 [1978]). We have now extended these studies to evaluate the role of intranasal irrigation with a large number of compounds. Initially, we monitored the effect on food-finding behavior. Many compounds (3-acetylpyridine, kainic acid, ethylmalonic acid, pronase, tyrothricin, Ellman's reagent, etc.) had little or no effect on food-finding behavior. However, intranasal irrigation with vinblastine SO4 (10 μmol) had effects similar to 0.17 M NaSO3 on behavior, olfac­tory bulb weight, S-100 protein, olfactory marker protein (OMP), synthesis and transport of carnosine (STC) and carnosinase ac-

**H.**

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 anaesthetia the baseline shift of the AEP disappeared and the oscillatory activity present in the olfactory bulb (OB) is not a true centrifugal input, the cell compound action potential. While the AAC interconnects the PON AEP without a decline in the amplitude of the mitral cell compound action potential. In contrast, tetanization of the AAC resulted in suppression of periods, with an increase in the amplitude of the baseline shift. In certain conditions 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 cell compound action potential.

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 activity present in the olfactory bulb (OB) is not a true centrifugal input, the cell compound action potential. While the AAC interconnects the PON AEP without a decline in the amplitude of the mitral cell compound action potential. In contrast, tetanization of the AAC resulted in suppression of periods, with an increase in the amplitude of the baseline shift. In certain conditions 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 cell compound action potential.


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 APN (N-(4-aminophenyl)maleimide) dianthracene staining technique (Sipple, 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 entirely block APN staining.

Since a 5 minute application of NEM abolished the EOG but did not block APN staining of SH groups, tritiated NEM (2uCi/nosiril) 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 H2-NEM. This will indicate the feasibility of mapping specific receptor sites in the epithelium using maleimides.
Thus, local cooling of the IXth nerve impaired both axoplasmic of labeled material proximal to the cooled segment. Furthermore, 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. Further, 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.

EFFECT OF PHOTOPHASE ON PRIMARY TASTE RECEPTORS. Elizabeth Onand* and Jacob Zabara, Dep't Physiol., Temple Univ. Health Sciences Center, Philadelphia, PA 19140.

Richter thought receptor changes could account for altered taste preferences, but Pfaffmann did not show any electrophysiological differences between normal and deafferentized rats. Furthermore, taste receptors do change. Butterfly larvae receptors have a decreased impulse frequency when their adequate stimuli had been present in ingested food prior to testing6. In mistreated blowfly adults, receptors show reduced impulse frequencies7. Fewer sensilla produce impulses during discharge in the blowfly7, and in the locust following feeding, when sensilla tip resistance is high6. We report similar multiple changes associated with phoshine in Musca domestica.

A microelectrode 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 P1 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 (0.3M sucrose), lighted animals showed the responses characteristic of animals tested with fewer impulse types, lower frequencies, and brief discharges. In addition to spike potentials, slow oscillations occurred 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 responses are related to the photophase as well as ingestion, is indicative of a broadly based regulatory system for taste receptor action.

Neural responses to amino acids in the rat. Thomas C. Pribramm* and Thomas B. 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 may be more complex than a simple preference for N and K, individual a.a.'s have been shown to be effective taste stimuli in humans and lower animals. We recently determined the neural effectiveness of 12 L-a.a.'s relative to 1 N 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 applications 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 +0.69 with their solubility in water. Thus, such a definition of responsiveness in 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 arbitrary defined as a change of 50% in spontaneous activity level. By this definition, the order of a.a. effectiveness was histidine > cystine > glycine > alanine > methionine > methionine > arginine > leucine > tryptophan > valine > isoleucine > glycine = threonine > proline. This sequence correlated +0.88 (p<.001) with that of human thresholds for the same stimuli. (Supported by NIH grant NS 10405.)
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 afferents are widely distributed to the same telencephalic structures as in macrosomatic rodents. Utilizing injections of the retrograde horseradish peroxidase (HRP) into the olfactory bulb of the rhesus monkey we 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 olfactory nuclear (AON) except the paras externa externa, paras lateralis externa, paras medialis externa, and paras medialis interna. AON and the primary olfactory cortex (POC) were labeled bilaterally while the medial transition cortex (MTC) between the paras medialis of the AON and the indusium griseum was labeled only ipsilaterally. The dorsoventral and mediolateral labeling was identical to others as part of the anterior hippocampal rudiment. In the POC labeled neurons were seen ipsilaterally in both layers I and II. Ipsilateral 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 and lateral amygdala area as well as superficially in the cortical amygdaloid nucleus. Labeled neurons were also found in both the vertical and horizontal limbic system such as the diagonal band, the medial prefrontal cortex, the 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 mammalian carnivorous and marsupial primates. The primate olfactory system does not show a progressive development, there is little anatomical subdivision of lateral entorhinal cortex. In the amygdala and the primary olfactory cortex (OCx) although some reports have described a preferential tufted cell projection to the OCx and perhaps to anterior olfactory nucleus (AON) (Baird, 1976; Price, 1977). Those reports emphasized labeling of small clusters of cells from all parts of the OB after localized injections of HRP into the OCx. 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 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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 percent 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.

Schwartz & Rowe (1977) have reported serious deficiencies, such as cannibalism, in maternal behavior of primiparous rats after bilateral olfactory bulbectomy (BOB). However, this could be due to lack of innervation of 1) the main olfactory system, 2) the accessory olfactory system, or 3) both. Fleming and Rosenblatt (1973) found that irritation of the nasal cavities with ZnO, sodium bicarbonate or formaldehyde (all of 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 hypothesize that denervation of the accessory system was primarily responsible for the deficiencies indicated by Schwartz & Rowe.

In order to test this, 19 female rats and their litters were observed from prenatal 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 normal controls (NC) were doing to verify VN. Surgery was performed before mating. 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 performed on Days 4, 7, and 10. No consistent differences were found among groups in this test. A number of other behavioral items (such as self-grooming, climbing or rising, and digging or burrowing in shavings) were measured, and the majority of times all pups were returned to the nest by the end of the test. A number of other behavioral items were measured 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 consistent differences were found among groups in these tests. These data indicate that an intact accessory system is not necessary for the development of adequate maternal behavior. The results of Schwartz & Rowe may due to a small population of the olfactory receptors. The stimuli used by Schwartz & Rowe are due to the presence of both main and accessory systems, 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.


Several approaches have been taken toward the behavioral classification of the gustatory system in the rat. Tatemichi et al. (1976) used techniques were by Erickson (olfaction and taste, 1963, 205) to examine taste similarities among stimuli in rats. Morrisson (Can. J. Psychol.) also developed techniques to generate taste quality profiles based on the generalization of a learned task from a conditioning stimulus to several others. The taste aversion test was used by Nowlis & Frank (olfaction and taste VI, 1974) to generate taste quality profiles of hamsters. Preference tests were done for combinations of stimuli which were then compared to single fiber sensitivities in the chorda tympani nerve. The present study provides a comparison between gustatory and olfactory systems. Histological sections from 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 similarity in terms of comparison to the licking rate following aversion. These cross-generalization measures were used to generate a similarity score for each pair of compounds. The similarity scores may be obtained by comparing all interactions to the other stimuli. These similarity profiles were then compared to the results of several types of analysis of neural responses: responses from central neurons, generalization of hamsters following conditioned taste aversions to each of these compounds, and parabacillary (PB) and parabacillary (PB) of the hamster. Cross-correlational profiles for each stimulus across all the others were directly comparable to the similarity score. A very good agreement between these measures for both the NTS and PB pontine neurons, with the exception of the correlations involving the PB pontine neurons. Therefore, the stimulus classification of gustatory quality, behavioral

269 BIOCHEMICAL BASIS OF THE SYNERGISTIC TASTE EFFECT OF MSG AND 5'-RIBONUCLEOTIDES. Emilio Torii* and Robert W. Cagnan. Monell Chemical Senses Center, Univ. of Pa., Veterans Admin. Hosp., Philadelphia, PA. 19104

Monosodium glutamate (MSG) evokes a taste sensation that is characterized as "savory" 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 quality 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 elucidated, although various candidates have been suggested. (R.H. Cagan (1977) In Chemical Senses and Nutrition, ed. by Kare and Hauser. 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[3H]glutamate to preparations from bovine circumventricular (CV) (taste) papillae and from tongue epithelium (EP) devoid of taste buds as a control. Differential centrifugation of homogenates of EP and of the sideward epithelium from CV resulted in a sedimentable fraction. Binding was assayed using L[3H]glutamate as a ligand with a rapid Millipore filtration method (J. M. Kruger & R. W. Cagan (1976) J. Biol. Chem. 251: 88-97).

Substantial binding occurs to CV, while the amount bound to EP is very low. The Kd for L-glutamate was estimated to be 17-20 nmol/mg protein, a value that is consistent with the Kd estimated from taste receptor cell membranes. The maximal binding capacity increased from 10 to 50 nmol/mg protein to 60 to 100 nmol/mg protein. The enhancement shows a degree of specificity for the nucleotide indicating that the effect is not merely a nonspecific perturbation. 5'-CMP, 5'-AMP, and IMP 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 seen in homogenates of EP. Guanine, 5'-GMP, and ATP were ineffective in enhancing glutamate binding to CV.

We postulate (i) that the synergistic effect between MSG and 5'-ribonucleotides may be due to interaction with the receptor for glutamate in the CV, and (ii) that the mechanism of the effect is an increase in the number of available glutamate binding sites caused by the ribonucleotides. Evidence may be related to the enhancement of binding seen with the alantime taste receptors of the catfish (R.H. Cagan (1977) Soc. Neurosci. Abstr. 3: 77, Abstr. 111). In addition, the stimulation of glutamate binding caused 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, MYRICIC, OLEIC AND LINOLEIC ACID, AMYL ACETATE, DIMETHYLBZYLCARBANYL ACETATE AND ANISOLE. Don Tucker and Sadao Kiyohara*. Biol. Sci., Florida State Univ., Tallahassee, Fl. 32306, U.S.A.

Olfactory nerve twig preparations were used for electrical recording of action potentials from single receptor cells. The overlapping impulse activity, due to the large numbers of about 0.2 um diameter axons contained in the sample, made it possible to count spikes from a population by counting the number of licks to each stimulus following aversive conditioning. The taste aversion that occurs following association of a stimulus with the aversive taste of sodium chloride was used by Nowlis & Frank (olfaction and taste VI, 1974) to generate a similarity score for each pair of compounds. The similarity scores may be obtained by comparing all interactions to the other stimuli. These similarity profiles were then compared to the results of several types of analysis of neural responses: responses from central neurons, generalization of hamsters following conditioned taste aversions to each of these compounds, and parabacillary (PB) and parabacillary (PB) of the hamster. Cross-correlational profiles for each stimulus across all the others were directly comparable to the similarity score. A very good agreement between these measures for both the NTS and PB pontine neurons, with the exception of the correlations involving the PB pontine neurons. Therefore, the stimulus classification of gustatory quality, behavioral

RESPONSIVENESS OF DOG OLFACTORY RECEPTORS TO THE FATTY ACIDS BUTYRIC, MYRICIC, OLEIC AND LINOLEIC ACID, AMYL ACETATE, DIMETHYLBZYLCARBANYL ACETATE AND ANISOLE. Don Tucker and Sadao Kiyohara*. Biol. Sci., Florida State Univ., Tallahassee, Fl. 32306, U.S.A.

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 changes in olfactory function and anatomical data from the granulopriival 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 naming 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, tests to reveal 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 novel visual and visual-tactile object 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 X-irradiation are not similar to reported effects of bulbectomy, arguing against a mass effect 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.


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 bud function such as 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 stimuli. Surface apical (SA) cells 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 MgCl₂, 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 MgCl₂, 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 cells in contact with Procion 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 are identified physiologically here correspond to the morphologically defined light and dark cells respectively. Responses of TB-1 cells imply a taste receptor function, whereas SE cell responses suggest secretory, supportive, and/or receptive function.

273 POSTNATAL NEUROGENESIS AND REGENERATION OF THE VOMEROSAL EPI- THELIUM FOLLOWING AXOTOMY IN GARTER SNAKES. Ruu-Tong Wang, Louis Guild* 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, Neuro-science Abstract, Vol. 3, p. 85) revealed heterogeneity in cell morphology particularly in both the undifferentiated (UD) and accessory (Ap) cell layer. Injection of 3H-proline into the 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 S07RR05401)


Frequently in taste neurophysiology the possibility of types of neurons corresponding in some sense with the "primary" taste qualities of humans has been entertained; recently types of gustatory neurons have been proposed by Frank according to which the classical "primary" stimuli single neurons give their best response (Ward, 1974; 241, 1977) are suggested to occur more or less continuous distribution of neuron profiles. This variation and previous research by Erickson (Psych. Rev. 75: 447, 1968) and associates suggest that the VN nerves of garter snakes (Thamnophis sirtalis & T. radix) may contain a single VN organ. Injection of 3H-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.

The VN nerves of garter snakes (Thamnophis sirtalis & T. radix) were bilaterally transected. 3H-thymidine was injected intra-cardially 24 h prior to sacrifice in animals surviving 1 to 28 days. 3H-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 Rp neurons on postoperative day 2. Degeneration marked by retrograde necrosis and cell loss in the Rp 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 Rp cell layer. Ud cells continue to increase in number and, by 4 postoperative weeks, occupy two-thirds of the original cell column.

Studies of 3H-proline have revealed 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 Rp cells at the anterior column of the cell body layer. The number of axons forming 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 3H-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.
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 mea-
sured by recording action potentials extracellularly are clearly 
evident between two and four days after the receptors first differ-
entiate from neural anlage 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 af-
fected 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. Re-
recording from single receptors was remarkably easy in fetuses 16 or 
more days old. In most preparations only a brief search was re-
quired to isolate units with either a platinized metal microelec-
trode 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, al-
though some units lasted for more than an hour. Responses to stim-
ulation were repeatable during this period. EOG amplitudes were 
stable for periods exceeding 5 hrs in the best preparations. Com-
monly the log of the EOG amplitude varied linearly with the log of 
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 umbilical 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% O2-
5% CO2. Supported by NSF Grant No. BNS 75-02339.

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, 
nearl 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 inges-
tion. Sustained ingestion completes the cycle as satiety super-
evanes.

The gain of the system relates to the development of the CES. 
The fully developed CES represents a state of autorhythmic ac-

tivity 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 stimu-
alization (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:

\[
\frac{dz}{dt} = -e + Zi + de \cdot \frac{dz}{dt} + Zi \cdot \frac{dz}{dt}
\]

where \( S_i \) is the central excitatory state (CES) 
\( d \) is the excitability factor 
\( e \) is excitation

This model, which represents an integration of receptor dynam-
ics with centrally mediated processes, will be discussed in rela-
tion to pertinent mammalian studies. (NIH Grant ROI-NS-14209-A).

Omand, E., Comp. Bioch. Physio., 38A: 265, '71; and Dethier, 
COMPARATIVE NEUROBIOLOGY

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 clarkii (Barrera-Mera et al., 1978) suggested that bilateral input of light activates the neural endocrine system of the sige gland in both eyestalks and thus modulates the crayfish retinal sensitivity. We have found that eye glow area (ERG stimulation, due to the activation of retinal pigments) can also be heterolaterally induced (Fig. 1 A & C). This response, better observed during the resting state 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 optic nerve (Fig. 1 D).

The equal migration toward the light adaptation position of the distal (6) and proximal (p) RSP. Since the ERG, EGA size and dRSP follow a similar time course we believe RSP mobilization is an important regulating influence on retinal sensitivity due to the action of light adapting hormone.

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 Pnak-Finkel methods. Seven to 10 animals were injected with 100 to 200 nl of [3H]-proline in the olfactory bulb and allowed to survive 7-12 days. The olfactory peduncle was cut in 3 animals who received 3H-proline injections.

The present analysis utilizes the nomenclature of Nieuwenhuyse (J. Hirnorrh. Soc. 6:171, 1963). The olfactory bulb projects to the ipsilateral subpallium, pal- lium, preoptic hypothalamus, and to intrinsic olfactory bulb. As the medial olfactory tract (MOT) courses along the ventromedial surface of the subpa- llium a sparse olfactory field appears lateral to the ventral subpallial nucleus (Vv). Fibers course dorsomedially to terminate in a capsular fashion about the dorsal subpallial nucleus (Dp) and the 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 anterior and induces to Dp also receives olfactory input. This area (Dv) also contains a dorsal region receiving a sparse olfactory input and a zone where the various afferent fiber systems with degeneration and/or autoradiographic methods, the thalamus of gars has been divided into two major olfactory subdivisions: the pars tuberculi posterior and the pars ventralis form longitudinal zones, whereas the pars tuberculi posterior lies caudal-ventral to the pallium, and gives rise to a commissure also. The LOT crosses in the AC and the habenular commissures.

These findings generally confirm those in the bullhead catfish (Finger, JNC 161: 125, 1975). Additional subpallial and preoptic inputs are described.

This work was supported by Rackham Dissertation Grant FUND, Univ. of Mich., and by PHS Grant 1 R01 EYO2485 to R. Glenn Northcutt.


We have found that in most vertebrates between two and ten percent of the basal metabolic rate is used by the CNS. The relationship is positively linear for the following animals: perch, trout, sharks, frogs, python, turtle, chicken, pigeon, primate, and cetaceae. Total 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 .99.

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 (Adams and Aisen, 1975), which may be due to recent phylogenetic changes 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 the log-log determinations show a correlation coefficient of .98 and a slope of .99. The regression line is a linear one, unlike the logarithmic equations which have been used. It is directly proportional to 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 an age or magnitude correction. 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°C.

In birds, two paths descend from the thalamus: a thalamo-cerebellar 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 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 crocodile, *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 paths most likely allow for a possible link 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 labeling 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.


From recent studies on visual system pathways, some common patterns can now be recognized within various groups. Figures below show general patterns of retinotectal projections in non-mammals (A) and mammals (B). While most non-mammals have predominantly crossed retinotectal projections, the tectum sends substantial connections to both the ipsilateral and contralateral thalamus. In mammals, however, the retinotectal projection is substantial ipsilaterally (Nebel et al.,'75; Graybiel,'76; Harting and Gullery,'76; Tiggges 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 tectencephalon 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 thalamotectal projections in shags, 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.

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 visual 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, a 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 ecostriatum, 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 ecostriatal lesions showed moderate to severe impairment on intensity and pattern problems.

After postoperative reacquisition of the discriminations, the ecostriatal-lesion group received lesions of the visual Wulst and the visual Wulst-lesion group received lesions of ecostriatum. In general, the effects of ecostriatal 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, ecostriatal 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 ecostriatal lesions resulted in a postoperative return to performance levels similar to those of birds with visual Wulst intact. In a third group, simultaneous lesions of visual Wulst and ecostriatum were made. These birds was equivalent to that of pigeons with visual Wulst lesions that had been made after ecostriatal lesions. The data indicate that visual Wulst lesions: i.e., the relative sparing of pattern discrimination following visual Wulst lesions depends upon an intact ecostriatum. No order effect was seen after ecostriatal lesions. Since the destruction of ecostriatum after visual Wulst lesions produces no greater impairment than lesions of ecostriatum alone, ecostriatum does not appear to serve as a 'surrogate visual Wulst' in birds with visual Wulst lesions.

REFLEX CONTROL OF POSTURAL MUSCLE STIFFNESS IN HERMIT CRAB. William D. Chappie. 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 superficial musculature (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 tone is elevated by the initial high frequency burst of the motoneurones and slowly declines during the after-discharge. The VSM are composed of four parallel groups of muscle fibres, 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 epididymis 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, CARASSUS 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). Sperm release was evoked at 61 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., 190, 215-232 (1961)) were used, except for the first fish (2 stimulation sites) the anesthetic level (0.15%) was half that used with sunfish. The "standard procedure" of testing an electrode area was used. As for serotonergic neurons in lamprey, evidence for the technique of "maximizing the response" by moving the electrode up and down was used for 12 sites. Since there appear to be no similar sites of stimulation in the two species, the data from each have been combined. Several low threshold sites (15-50 µA) were found in just dorsal to the nucleus glossopticus, whereas about 1/10 sites required between 101-200 and points (51-100 µA) were scattered throughout this region as well as posterior to the dorsal hypophysis, submammillary area and ansalateral medulla (see table below). Allowing for species differences in brain anatomy, the results in goldfish appear similar 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 are shown in table 1.

<table>
<thead>
<tr>
<th>Region stimulated</th>
<th>Threshold (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoptic and adjacent areas</td>
<td>0 0 0 5 4* 1</td>
</tr>
<tr>
<td>Hypothalamic-near post-optic commissures</td>
<td>0 1 1 1 10</td>
</tr>
<tr>
<td>Submammillary area</td>
<td>0 0 0 3 2</td>
</tr>
<tr>
<td>Tegmentum-near the n. glossopticus complex</td>
<td>4 3 0 1 1*</td>
</tr>
<tr>
<td>Basalateral medulla-cerebellar to medullary lobe levels</td>
<td>0 2 1 0 2</td>
</tr>
</tbody>
</table>

*one site was tested using the higher anesthetic level


The response to iontophoretically applied acetylcholine (ACh) was observed by intracellular recording from heart muscle fibers of the three species of bivalves: Mytilus edulis (Clinical), Mercenaria mercenaria (clam), and Crassostrea virginica (oyster). This species were chosen as representatives of the different types of responses to bath-applied ACh (excitation-inhibition). A few data points for each species were obtained from fish spawned spontaneously. Heart fibers were impaled with 80-100 MA microelectrodes filled with 2M KCl-1M KCl or with 3M KCl in the medium and superfused with ASW. The fibers were exposed to 20-50 HA iontophoretic microelectrodes filled with 0.1 M AChCl. In Mytilus, iontophoretic microelectrodes were filled with 0.1 M AChCl. In Crassostrea, iontophoretic microelectrodes were filled with 0.1 M AChCl. In Mercenaria, ACh caused only a relatively slow hyperpolarization and never 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 mediated by the existence of acetylcholine, to which the depolarizing response was much less sensitive. The depolarizing and hyperpolarizing responses were both accompanied in an apparent fashion by a decrease in spontaneous activity, as determined by passing short pulses of current through an extracellular suction electrode. In Na-free ASW (substituted with Tris or glucose-ammonium) the depolarization response disappeared reversibly. In K-free ASW the hyperpolarization response increased and in elevated-K ASW (22, 45 and 67 mM) it decreased progressively. The highest concentration of potassium reversed the K-free depolarizing response, even though the resting membrane potential was significantly depolarized. In CI-Free ASW (substituted with Methylsulphate and sulphate), 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 responses 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.


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 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 ontogenesis. 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., 1972, 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. The present study was undertaken to investigate the other major class of cyclostome, Myxine glutinosa. (Hagfish).

Serotonergic neurons were identified by retrograde labeling using the horseradish peroxidase technique. HRP labelled cells were found in the rostral dorsolateral tegmentum, in the torus semicircularis and in the nucleus lateralis. A large group of cells caudal and rostral to the nucleus glomerulosus also projects to the tegmentum. A number of HRP labelled cells were found in the group of efferents that exit the lateral tegmentum. Some of these cells appear to have one process that reaches the tegmentum and another that continues down the ipsilateral ventral root in the spinal cord. These cells were also found in the lateral tegmentum, several diencephalic-pretectal nuclei, the contralateral tegmentum and a number of tectal centers. The diencephalic-pretectal areas include the area pretectalis, nucleus pretectalis, nucleus dorsomedialis and nucleus dorsosoralis.

HRP labelled cells were found in the rostral dorsolateral tegmentum, in the torus semicircularis and in the nucleus lateralis. A number of HRP labelled cells were found in the group of efferents that exit the lateral tegmentum. Some of these cells appear to have one process that reaches the tegmentum and another that continues down the ipsilateral ventral root in the spinal cord. These cells were also found in the lateral tegmentum, several diencephalic-pretectal nuclei, the contralateral tegmentum and a number of tectal centers. The diencephalic-pretectal areas include the area pretectalis, nucleus pretectalis, nucleus dorsomedialis and nucleus dorsosoralis.

The optic tectum in fish has long been considered a primary site of sensory motor integration. Recent studies in several teleosts have suggested that the majority of tectal afferents are those from the retina and tegmentum. The only afferents to the tectum that have been studied with modern techniques are those from the retina and tegmentum. 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 tegmentum, several diencephalic-pretectal nuclei, the contralateral tegmentum and a number of tectal centers. The diencephalic-pretectal areas include the area pretectalis, nucleus pretectalis, nucleus dorsomedialis and nucleus dorsosoralis. The optic tectum in fish has long been considered a primary site of sensory motor integration. Recent studies in several teleosts have suggested that the majority of tectal afferents are those from the retina and tegmentum. The only afferents to the tectum that have been studied with modern techniques are those from the retina and tegmentum. 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.

Fibers of passage through an injection site often pick up HRP. If the fiber fails to pass through the tegmental projection area it enters the contralateral tegmentum and out into the contralateral torus semicircularis, where they form terminal fields. These fields appear to be the primary site of sensory motor integration.

Gangliosides are sialylglycosphingolipids which separate chromatographically according to molecular complexity into patterns that vary phylogenetically. Since a regionally specific pattern of gangliosides has been reported for the mammalian CNS, particularly differences between the retina and the brain, the observed phylogenetic variation 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).
CEREBELLAR EVOLUTION IN CARTILAGINOUS FISHES. R.

of retia in other orders of mammals in different ecological

walls of the arteries of the spinal rete but relative lack of

ous bony channels for the heart and brain during dives. We found

and functional studies of the retia so we can understand how such

sense to the anterior and middle cerebral arteries were seen. The

and optic nerves. Intracerebral arteries then emerge from this

space of the spinal canal. Bilateral spinal meningeal arteries

formed to a fibrotic strand in the tympanic cavity. This vessel

cerebral blood supply of the dolphin passes through the massive

stratum and medial pallium) in the forebrain of the bullfrog.

To elicit a response from these regions, like the auditory

thalamus, 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 the auditory cortical areas. 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.)

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 squatiniforms, and in other ceratodontids, Scyllichthius, Gymnura, and most rajiform skates.

The corpus has hypertrophied, resulting in a foliated condition, which in many species has become 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 help maintain a normal pressure in pressure-sensitive 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 aquatic mammals, 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.)

ELECTROPHYSIOLOGICAL EVIDENCE FOR AUDITORY RESPONSIVE AREAS IN THE DIENCEPHALON AND TELENCEPHALON OF THE BULLFROG, RANA CATESBEIANA. Karen M. Hudspeth and Robert B. Capranica, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

In a recent anatomical study Neary (Am. Zool. 12: 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 hierarchical processing of vocal signals and other complex sounds in the central auditory system of amphibians.

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 the auditory cortical areas. 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.)


Canary song is a learned behavior characteristic of adult males during the spring season. Song is produced by the syrinx, an organ innervated by axons from the hypoglossal nucleus. These neurons 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 "shell" of neostriatum outlining the medial and ventral borders of HVC (Kelley and Nottebohm, Brain Res. 10021) and D.B. Kelley (Dept. Psychol., Princeton Univ., Princeton, NJ 08540).

The present study investigated afferent connections of HVC and RA by means of the retrograde tracer, horseradish peroxidase (HRP) and tritiated adenine. 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 ventral-caudal edge of field L projects to a "cup" of tissue apposed to rostroventral RA. Nucleus 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 to the magnocellular nucleus of the anterior neostriatum. Cells in NIF are larger than those in adjacent field L; diameters range from 12 to 20 u. Nucleus MAN has been shown to contain antigen-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 the NIF, MAN, and telencephalic vocal control nuclei function in the control of vocal behavior.

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. Neurol., 1976) failed to find any projection to the lateral geniculate nucleus (LGN). These authors suggested that retina-thalamo-telencephalic 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 to their connections (afferents). Therefore, a retino-thalamo-teleencephalic system in teleosts might not involve the nucleus herebefore 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 contra-lateral 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 fibres 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.

---


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 monomeres. 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 neurotrophic influence of the nucleus of the solitary tract.

(Supported by NIH Fellowship MH 05390)

---

SOCIETY FOR NEUROSCIENCE

---


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. Neurol., 1976) failed to find any projection to the lateral geniculate nucleus (LGN). These authors suggested that retina-thalamo-telencephalic 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 to their connections (afferents). Therefore, a retino-thalamo-teleencephalic system in teleosts might not involve the nucleus herebefore 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 contra-lateral 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 fibres 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.
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 subretinal 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 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 axon 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 cortex, 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 overriding the hypothalamic input described by Neary and Wilczynski (Anat. Rec., 187: 665, 1977). The cells responsible for the preoptic projection 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 tectum.

This work was supported by Rackham Dissertation Grant funds, Univ. of Mich., and by PHS Grant 1 RO1 EY02485 to R. Glenn Northcutt, Division of Biological Sciences, Univ. of Mich.

RETINOFOCAL PROJECTIONS IN THE RED-BACKED SALAMANDER: EVIDENCE FOR AN IPSILATERAL RETINO-TECTAL PATHWAY. H. Zakon. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

The projections of the optic nerve in Plethodon 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 (VLM) and more dorsally in the neuropil of Bellonci (BN). Ipsilateral projections to the VLM display less dense degeneration and extend 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 contralateral 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 retina-tectal projections have been described in 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 retina-tecal pathway, the direct retinal origin of this pathway is unclear. This study uses a technique which has no transsynaptic effects at these survival times, verifies the existence of a direct ipsilateral retina-tecal pathway.
DEVELOPMENT AND AGING

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 observation 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%.

Therefore, lead-exposed animals are more slowly growing fibers (lateral perforant path) resulted in more slowly developing animals and in hypotrophic regenerating 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 old) values at birth and d 5 in DA 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 TH activity increased to 124% (p < 0.001) of adult values by d 24. Enzyme activity was decreased more slowly to adult levels by d 30-60. By immunostaining with specific antibodies the overshoot of TH activity in 5HT and TH activity in raphe was maintained and the concentration of protein remained constant.

HEMISPHERE, 2, 261-255.

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 92122

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 concentrations of total enkephalins and enkephalins during pre- and postnatal development in the rat. Although the absolute amounts of both endorphin and enkephalin increase with age, increasing severalfold by adulthood, the increases are not consistent in all the regions studied (see Table I), the concentrations, expressed on a protein basis, reveal interesting differences. Between embryonic day 12 ED20) and the neonatal day 6 (PN6) days both endorphin decreases in all regions; 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 change from ED20 to PN6 in any of the regions studied; enkephalin concentration remains almost constant after birth except for a marked increase (about 3-fold) in the region containing the pontine and superior cerebellar areas and the substantia nigra (SN).

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

<table>
<thead>
<tr>
<th>Age</th>
<th>Enkephalin Units*</th>
<th>Endorphin Units*</th>
<th>Brain Protein (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED-20</td>
<td>1.3 ± 0.2</td>
<td>6.4 ± 0.8</td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td>PN-6</td>
<td>7.4 ± 0.5</td>
<td>11.2 ± 1.3</td>
<td>48.5 ± 1.8</td>
</tr>
<tr>
<td>PN-25</td>
<td>40.7 ± 2.5</td>
<td>76.7 ± 2.4</td>
<td>263.1 ± 16.5</td>
</tr>
</tbody>
</table>

*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 Leu14-His27 segment and cross reacts 100% on a solar basis with both β-LPH and the 31K protein. The Leu-enkephalin RIA shows 3X cross-reactivity for Met-enkephalin. Results are expressed as the mean of 3-4 determinations ± the standard error of the mean.
PHOSPHORYLATION AND AGING: cGMP DEPENDENT PROTEIN KINESINES IN HUMAN STRIATUM. Diethelm H.Bohme, and Neville Marks. The V.A. Hospital East Orange N.J., 07019 and Institute for Neurochemistry and Drug Addiction, Wards Island, N.Y., I0025.

Altersations 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 rat. Synaptosomal membranes were prepared from human tissues 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 of synaptosomal proteins changed with age in membranes from the diverse effects of agents known to alter cGMP levels or dependent protein kinases in vivo and may be linked to the ageing process.

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 day old wild type 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%, elongated neurites from the ganglia were cultured in the absence of added nerve growth factor. The activity of tyrosine hydroxylase (T-OH) increased 6-fold by 24 hrs. in control explants, but failed to rise in the presence of actinomycin-D. Ganglia explants also elaborated neurites for at least 6 hrs. in the presence of concentrations of cycloheximide or puromycin which inhibited protein synthesis. However, blockade of a 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 (Ar-C), an inhibitor of DNA synthesis. Blockade of DNA synthesis resulted in the virtual absence of support cells. However, neurons elaborated abundant neurites and in treated explants were associated with support cells. Moreover, T-OH activity increased 3-fold in the presence of Ar-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.

(With this work was supported by the NIH, the Dysautonomia Foundation Inc., the NSF and the Hirschl Trust Fund.)
MORPHOMETRIC STUDIES ON AGE CHANGES IN VISUAL CORTEX AND HIPPOCAMPUS IN THE RHESUS MONKEY. Kenneth R. Brizzee, Neurobiology Department, Delta Regional Primate Research Center, Tulane University, Covington, La. 70435

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 0.1% OsO₄, embedded in Epon sectioned at 4 µm and stained with toluidine blue.

The medio-lateral 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 µm in young adults to about 180 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.

MORPHOMETRIC 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-b-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 13.5 days of gestation (35-35 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.

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 peak and trough periods support a number of possible transmitter mechanisms develop in close temporal proximity in the differentiating neuroblast.

This work was supported by the NIH, the Dysautonomia Foundation Inc. and the DGMR, France and the Hirochi Trust Fund.

ONTGENETIC 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-b-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 13.5 days of gestation (35-35 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.

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 peak and trough periods support a number of possible transmitter mechanisms develop in close temporal proximity in the differentiating neuroblast.

This work was supported by the NIH, the Dysautonomia Foundation Inc. and the DGMR, France and the Hirochi Trust Fund.

DEVELOPMENT AND AGING

DELAYED DEVELOPMENT OF THE INPUT-OUTPUT ORGANIZATION FOR KITTEN MOTOR CORTEX. J. C. Bruce* and W. G. Tatton. Fac. Med., Univ. of Calgary, Calgary, Alberta, Canada T2N 4N2

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 on chronic-prepared kittens 9 to 67 days of age. Recordings were made from 41 cortical neurons while the contralateral forelimb was displaced at random intervals by a tendon stimulator as to rotate the elbow and stretch triceps brachii. DMG recordings were made from triceps in response to the displacements and to intracortical and immediately subcortical microstimulations. Average EMG activity was calculated for the ARH response peaks. The findings were: 1) SE values for responses in primary somatosensory cortex (areas 1 and 2) were in the range found for adult and exhibit adult-like values. Resting SE latencies were longer (25-28msec) than those in adults (10-12msec), in keeping with the lower peripheral 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 43-45 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 of age but latencies 5-6 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 responses 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 each peak. By 50-60 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 immediate forelimb motor cortex does not show adult-like responses until about 45 days. The development of mechanoreceptor input to motor cortex lags the development of effective output from the motor cortex to alpha motoneurons and occurs at the same interval as the appearance of adult-like EMG responses to forelimb displacements.

ICB is a Dysautonomia Foundation Fellow.


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 peripheries 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 day 21 fetal rat superior cervical ganglia were prepared on collagen (Bormstein, '82). Poly-L-lysine (50 µg/ml) prepolymerized 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 unless the clot is grown 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.)
EXPOSURE TO ETHANOL IN UTERO HAY DELAY MATURATION OF HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION IN THE RAT. Beatrice Cooley-Hathaway and Anna H. 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 rhythmity. Treatment with corticosterone or ACTH during the neonatal period leads to persistent alteration of some characteristics of adrenocortical secretion, suggesting permanent modification of the central neural substrate implicated with regulation of adrenocortical activity. Since ethanol has been shown to activate the adrenocortical axis 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 adrenocortical 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 ethanol 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.


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 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 end-plate 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 12 and processed routinely for paraffin sectioning. Cell counts were made by counting cells with nucleoli in alternate sections stained with hematoxylin. The extracellular muscles were frozen and processed for thin. 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 A2 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 reductions 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)


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, the rate of ne deprivations, 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 effect of age but to the stress of deprivation. Our laboratory has shown that treatment of neonatal rats with lithium chloride (LiCl) causes a long lasting steroid elevation in all ages. Since the injection of LiCl during conditioning caused a long lasting steroid elevation in all ages, and the brains of neonatal animals are faster than in the brains of adult animals, 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 [3H]NaHCO3, 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 (P2) fractions 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 rates is slower under conditions of rapid growth. (This research was supported by NIH Grant DA 00697).

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 effect of age but to the stress of deprivation. Our laboratory has shown that treatment of neonatal rats with lithium chloride (LiCl) causes a long lasting steroid elevation in all ages. Since the injection of LiCl during conditioning caused a long lasting steroid elevation in all ages, and the brains of neonatal animals are faster than in the brains of adult animals, 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 [3H]NaHCO3, 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 (P2) fractions turned over at 70% of the adult rates, whereas proteins in the microsomal fraction turned over at 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 rates is slower under conditions of rapid growth. (This research was supported by NIH Grant DA 00697).
ALTERATIONS IN PITUITARY-ADRENAL ACTIVITY OF RATS TREATED WITH ACTH1-24 NEONATALLY. Susan H. Dry-Keuss, Berriyln J. Branch*, and Anna N. Taylor. Dept. of Psychol. and Anat., Brain Research Institute, UCLA, Los Angeles, Ca. 90024.

Early life experiences are known to exert profound effects on endocrinoactive properties. We were therefore interested in studying whether neonatal hormone levels influence this phenomenon. In a previous study (Lorenz et al., Fed. Proc. 32: 296-299, 1973), we observed that long-Evans rats treated with ACTH1-24 (Armour, 4 IU/10g b wt) on days 7-9 exhibited significantly lower responses to restraint stress as compared to control animals. In the present study, Sprague-Dawley rats were injected sc with ACTH1-24 (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±0.09 days vs. 15.82±0.15: p<0.001).

In adults, animals were subjected to stress and jugular 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 response. 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 finding. Males treated with ACTH showed significantly less elevation of plasma B in response to stress in the peak period than did vehicle males (62±15% [SEM] vs. 124±91%). These same animals had smaller adrenal glands than controls. 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 ACTH1-24, s.c., 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 ACTH. This suggests that the effects of stress responsiveness do not represent hyper-responsiveness of the adrenal to ACTH. Since we have previously shown that exposure of rats at this age to ACTH 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.


Specific motoneuron nuclei innervate particular muscles selectively in the developing chick hindlimb. Each muscle is innervated by a coherent group of motoneurons in a characteristic position in both the rostro-caudal and medio-lateral axis normal) were done at stage 17 (Hamburger and Hamilton, 1955). 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. Functional motoneurons innervating muscles derived from the DM were situated laterally and those innervating the VMMs 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 can be an important topographical fashion according to position.

Superficially, lesions in this and other laboratories have shown that motoneurons are capable of innervating muscles to which 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 VMMs or DM, dorsal-ventral rotations (deviating the anterior-posterior axis normal) were done at stage 17 (Hamburger and Hamilton), prior to motoneuron outgrowth.

Histological sections at stages 25-36 showed that the medullar motoneurons still innervate the VMMS, even though this is now in a different position. Similarly, lateral motoneurons still innervate the DM. These results suggest that the limb bud is capable of affecting motoneuron projection patterns to result in selective innervation of DM and VMMS by lateral and medullar 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.
**SOCIETY FOR NEUROSCIENCE**

**324 RETINAL SYNAPTIC ARRAYS OF LARGE AND SMALL GOLDFISH.**


The retina of goldfish grows, as the goldfish itself grows, continually throughout its life. During growth, the inner nuclear layer (INL), the inner photoreceptor 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 micras, conventional density was 1259.1 for small fish and 1648.6 for large fish while ribbon density went from 29.2 for small to 17.91 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 IPL nuclei (synapses per amacrine or bipolar cell), planimetric density of ribbons, and visual field (synapses per degree of visual field).

The data so analyzed indicate that bipolar and amacrine synapses differ in their strategies of continuing synaptic 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

**326 ONTOGENY OF [C14]-2-DIOXYGLUCOSE UPTAKE IN THE RAT BRAIN WITH SPECIAL REFERENCE TO DAY-NIGHT DIFFERENCES IN THE SUPRAHYPOTHALAMIC NUCLEI.**

J. L. Bock and Robert W. Gourley, Dept. of Anatomy and Div. of Neuroscience, Univ. of California at San Diego, La Jolla, CA 92037.

The regional pattern of [C14]-2-deoxyglucose uptake (DG) in the adult rat brain has been shown by Schwartz and Sharp (J. comp. Neurol. 177: 355-360, 1978). In addition, Schwartz and Gainer (Science 197: 1089-1091, 1977) have shown a clear day-night difference in DG 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 [C14]-DG 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 DG distribution. Between days 1 and 21 there is a differential maturation of cerebral metabolism from one brain area to another as demonstrated by the DG method. The day-night differences in DG uptake in the SCN appear to be present by day 1 and are marked by day 10. This observation suggests that the circadian rhythms are already present in early 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 AGING IN MOUSE BRAIN: CHANGE IN ASTROCYTE POPULATION.**


Most information on changes in glial cells with age has been provided by electron microscopic and autoradiographic analysis of random sections of brain. 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 populations of glia 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 identically discontinuous sucrose gradients. This age-related difference in glial cell population was restricted to the brainstem and telencephalon, and not observed in cerebellar preparations. Repeated experiments confirmed that the alteration in banding density was associated with astrocyte-like cells. The population of astrocytes from young and old animals were readily characterized by their different banding 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 buoyant density, may indicate major age-associated changes at the biochemical level. Supported by NIH Grant NS 12334.

One hundred eighty-five Zivic-Miller rats, reared in either a restricted or enriched postweaning rearing 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 hyperactive, and over subsequent testing increasingly responsive, suggesting a failure to habituate. In Exp. 4, hungry Ss were not observed 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 a less active state. Restricted Ss did not respond as well as enriched Ss. In Exp. 5, locomotor hyperactivity 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 repetitive pattern of rewarded response when it is subsequently punished.

In tests of step-down and escape avoidance (Exp. 6), restricted Ss were shown to be considerably deficient in the ability to passively avoid nocuous stimulation, or to control and direct their behavior so as to escape electric shock. Moreover, restricted Ss showed increased latency of responding 2 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.
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. 92039.

We are investigating the differentiation of a class of identified neurons from a single identified neuroblast in embryos of the grasshopper Schistocerca gregaria, 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 descendents 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 neurons develop from specific DUM daughters along the left and right chains. By injecting Lucifer Yellow into growing neurons, we have found that the dorsal unpaired median neuroblast, with a single asymmetric left and right chains contain different types of neurons; the cells on the left extend laterally out peripherally, with RIA data. The SON of 21-day neonatal rats (regardless of sex) demonstrated the ultrastructural correlates of heightened vasopressin on each subsequent day until shortly after parturition when neuroblasts contained 63 ± 2 pg AVP/animal. There was approximately a 3- to 4-fold increase in immunoreactive vasopressin in each segmental chain of the developing brain. 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 thickness of their plasma membranes, it suggests an absolute loss of axo-dendritic synapses with advanced age. This synaptic loss is also suggested by the substantial age-related decrease in 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-dendritic synapses contributes to the process of age-related partial deafferentation of neurons in the rat dentate gyrus.

Supported by USPHS Grants RR-05403, AM-19761, NS-00259.
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 individual song nuclei coalesce and increase in volume, yet as late as 20–25 days post-hatching the sexes are equivalent. By 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 achieves 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.

The presence of intramembrane Schwann cells has been observed following x-irradiation of lumbosacral spinal cord of three-day-old rats (S. A. Gilmore and D. Duncan, Anat. Rec. 160: 675, 1968). In the present investigation intramembrane 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 thoracic and lumbosacral spinal cord (T/L) were irradiated. All these animals received a single exposure to 1760 rads.


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 individual song nuclei coalesce and increase in volume, yet as late as 20–25 days post-hatching the sexes are equivalent. By 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 achieves 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.

Development is studied in three groups of rats irradiated at three days post-irradiation. The accumulation of these cells in the mid-thoracic region was more extensive in the T/L only group than in the T only or L only group, 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.)

The presence of intramembrane 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 intramembrane 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 1760 rads.


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 individual song nuclei coalesce and increase in volume, yet as late as 20–25 days post-hatching the sexes are equivalent. By 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 achieves 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.

The presence of intramembrane 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 intramembrane 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 1760 rads.


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 individual song nuclei coalesce and increase in volume, yet as late as 20–25 days post-hatching the sexes are equivalent. By 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 achieves 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.

The presence of intramembrane 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 intramembrane 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 1760 rads.


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 individual song nuclei coalesce and increase in volume, yet as late as 20–25 days post-hatching the sexes are equivalent. By 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 achieves 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.
336 DEVELOPMENT OF TRIGEMINAL MOTOR NUCLEUS IN CHICK EMBRYO: LIGHT MICROSCOPIC OBSERVATIONS. Marietta S. 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 15. 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 root can be seen exiting the brainstem. The temporal sequence of ganglionic development 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, 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 cells and those of migrating cells are well demonstrated. These observations indicate that processes of neuron-1 of the operated side are dendrites originating from Interneuron-1 of the operated side are usually ipsilateral, cross the midline to terminate in the contralateral VAN. 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.

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 by stage 38, and motor neuron targets are defined 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 muscle 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 muscle are born from stages 19-22. In both thig 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 motor neurons which are born during different days of the motor neuron birthdate of ventral muscle mass origin. Limb muscles derived from the dorsal muscle mass (semimembranosis, post. iliotibialis, peroneus and triceps brachii) are innervated by more lateral motor neuron clusters which newborn during the motor neuron birthdate of dorsal muscle mass origin. 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 neuromuscular 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 NIMH grant MH-27677.

338 ABSENCE OF AUDITORY AFFERENTS ALTERS THE GROWTH PATTERN OF AN IDENTIFIED AUDITORY INTERNEURON. Ronald Hoy*, George Casaday*, Sharon Rollins*, (SPONT: H. Howland) Cornell Univ., N8AB, Langmuir Lab, Ithaca, NY. We have studied the effect of the absence of auditory afferent axons during postembryonic development on the adult morphology of the auditory system in the cricket Teleogryllus oceanicus. The ear, on the tibia of the foreleg, differentiates wholly during postembryonic development. The tibia is a lateral appendage formed after hatching and subsequent amputation of the regenerating tibia prevented the development of any auditory afferent fibers on the operated side. In sham-operated animals and on the normal side of unilaterally amputated animals (1) the lateral dendrite on the operated side is usually ipsilateral, (2) several 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. "Multicursive" (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 multicursive 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 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 PHR RR 07030 and the University Research Board.

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 15. 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 root can be seen exiting the brainstem. The temporal sequence of ganglionic development 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, 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 cells and those of migrating cells are well demonstrated. These observations indicate that processes of neuron-1 of the operated side are dendrites originating from Interneuron-1 of the operated side are usually ipsilateral, cross the midline to terminate in the contralateral VAN. 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.

339 POSTNATAL DEVELOPMENT OF BAHRIAN PREOPTIC AREA: A GOLGI STUDY. Chia-Bing Hsu*, C. Sue Carter, and William T. Greenough. Dept. 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. "Multicursive" (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 multicursive 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 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 PHR RR 07030 and the University Research Board.
DEVELOPMENTAL CHANGES IN GANGLIOSIDE COMPOSITION OF HIPPOCAMPUS, RETINA, AND OPTIC TECTUM. Carol Irving* and Louis Irving. Dept. Biochem., E. R. Shriver Center, Waltham, WA 02154.

The progressive emergence during development of precise cellular laminations and a topologically organized circuitry in regions such as the hippocampus, retina, and optic tectum many vertebrates ideally suits these regions for a study of chemical correlates of differentiation and positional coding in the nervous system. We have initiated methods for the extraction and analysis of gangliosides in order to correlate changes in the quantity and molecular heterogeneity of these glycosylated glycolipids, in response to the neurochemical 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 chlorormethanol:water (10:10:3), quantitated 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_{12}) 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 retina dentata), all the major ganglioside components were present in small amounts. Slight variation in ganglioside content and pattern within subregions of the hippocampus and retino-tectal 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)

342 ULTRASTRUCTURAL STUDY OF MORPHOGENESIS IN THE AUDITORY SYSTEM OF CHICK EMBRYOS: NUCLEUS MAGNOCELLULARIS. Jaiver*, Sonal, and D. E. 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 cochlear sensory endings and their target cells was studied with electron microscopy of perfusion-fixed brains from embryonic day 12 (E12) to hatching. E12-15: 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 adherent 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 presynaptic cytoplasm.

E16-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. These develop into small vesicles in extent until it is found associated only with somatic spines. Conclusions: Primary cochlear fibers initially contact distal parts of the somatic processes of 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 transition from non-functioning, flocculent material of the postsynaptic regions in the formation of synapses.

Supported by USPHS grants 7 RO1 NS13463 and 7 RO1 NS14354, and the Jeffries Wyman Fellowship.


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 the same rat, however, show developmentally young implants (up to 4 weeks survival) integrated neurons have certain characteristics of immaturity, such as sparse somatic and hair-like dendritic apical appendages and the presence of 3H uptake after transplantation by unlabeled graft tissues. These results indicate that embryonic tissue continues differentiation after transplantation and develops connections with the host nervous system. Such connections can persist for at least 4 weeks, even though the transplant may be situated in an abnormal site. (Supported by USPHS grant KO-01950 from the National Institutes of Health.)


We studied the effect of transplantation on the development of the cortex by grafting portions of the dorsal telencephalic telencephalic part of rat embryos of gestational ages E15-E22 (birth at E22) to the cortex of newborn rats. Embryonic graft tissue was labeled with 3H, followed by transplantation and grown for 1-8 weeks in the host.

The detection of newborns yields indicates 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 between the implant and the host tectum (or pretectum). This fiber bundle contains, at least in part, different axons from the implant.

Golgi impregnation of explants showed that variations of the two main classes of known cortical neurons, namely pyramidal and monopolar (e.g., stellate) forms, can be recognized in the implant. Pyramidal cells have a large single dendrite which seems to correspond to the afferent dendrite of the embryonic cell. In embryonic young implants (up to 4 weeks survival) integrated neurons have certain characteristics of immaturity, such as sparse somatic and hair-like dendritic apical appendages and the presence of 3H uptake after transplantation by unlabeled graft tissues. These results indicate that embryonic tissue continues differentiation after transplantation and develops connections with the host nervous system. Such connections can persist for at least 4 weeks, even though the transplant may be situated in an abnormal site. (Supported by USPHS Grant KO-01950 from the National Institutes of Health.)

Tissue cultures of sympathetic neurons were studied to determine the mechanisms involved in the establishment of adrenergic and cholinergic properties. Explants of early neonatal sympathetic neurons in culture were able to maintain adrenergic characteristics, while dissociated cultures were able to acquire cholinergic function. The development of this technique for culture of dissociated adult neurons. Dissociation was most successful using a 1 hour incubation of small SCG chunks in 0.25% collagenase (Worthington). Explants taken from the same rat, however, show developmentally young implants (up to 4 weeks survival) integrated neurons have certain characteristics of immaturity, such as sparse somatic and hair-like dendritic apical appendages and the presence of 3H uptake after transplantation by unlabeled graft tissues. These results indicate that embryonic tissue continues differentiation after transplantation and develops connections with the host nervous system. Such connections can persist for at least 4 weeks, even though the transplant may be situated in an abnormal site. (Supported by USPHS Grant KO-01950 from the National Institutes of Health.)

Direct biochemical analyses were used to examine the appearance of synaptic junctional (SJ) macromolecules during the formation of asymmetric synaptic complexes in situ. The differential degrees of similarity was observed in the composition of major proteins in SJ fractions obtained from immature brain at various stages of postnatal development by subcellular fractionation methods previously developed for mature nervous system tissues. Electron microscopy on Oso4 and E-PTA stained synaptic fractions revealed that immature SJ's are morphologically similar to those isolated from adult SJ's. SJ's isolated from immature rats brains displayed easily recognizable post synaptic densities (PSDs). SJ's isolated from immature brains (0-5 days of age) were smaller and their PSDs apparently incrustation formed. SJ fractions from immature animals contained a higher proportion of post synaptic membrane specializations (post synaptic membrane plus PSD) relative to intact SJ's than did adult SJ fractions. The protein yield in SJ fractions increased 4-fold during development, a change that paralleled the temporal appearance of asymmetric synaptic complexes in situ.

The protein and [125I]Concanavalin A (Con A)-binding glycoprotein compositions of SP 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 proportions of these proteins were constant throughout development. The Con A-binding glycoproteins, which are localized to the external surface of the postsynaptic membrane overlying the PSD, are present in similar quantities in immature (4-16 days) and adult SJ fractions. However, large differences in the composition of Con A-binding glycoproteins were observed in SP fractions during postnatal development. An exception to these developmental similarities was the major FSD protein which, when compared to adult SJ, was present in SJ and SP fractions at approximately the same levels throughout the period of active synaptogenesis (8-18 days postnatal).

These results show that post synaptic membrane glycoproteins were present in Con A-Sepharose 4B columns 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 basic adhesion. In contrast, the major FSD 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.)

Astromcytic cell lineage. An in vitro study. R. H. Juurlink, S. Fedoroff and L. Hertz. Departrnent of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, 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 immune system of mice show different patterns at different 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. Younger lesioned rats (1) undergo a spectrum of morphological changes. In vitro experiments have indicated that the A type colonies develop into the mature astrocytes containing the type B colonies, which in turn transform into the type C colonies (1). The latter react to addition of the dibutyl cyclic AMP by intense formation of processes localized to the cell bodies and thin processes in the extracellular space.

In order to determine the precise origin of the cells which form type A colonies, the subventricular and cortical regions of newborn (PO) newborn (PO) and adult hamsters, were studied. From each region, cultures were obtained using subventricular and cortical areas had less than half the number of type A colonies than cultures of C0 mice and twice as many type C colonies. These findings supported the hypothesis that immature (4-14 days) and adult SJ fractions. However, large differences in the composition of Con A-binding glycoproteins were observed in SP fractions during postnatal development. An exception to these developmental similarities was the major FSD protein which, when compared to adult SJ, was present in SJ and SP fractions at approximately the same levels throughout the period of active synaptogenesis (8-18 days postnatal).

The protein yield in SJ fractions increased 4-fold during development, a change that paralleled the temporal appearance of asymmetric synaptic complexes in situ.

The protein and [125I]Concanavalin A (Con A)-binding glycoprotein compositions of SP 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 proportions of these proteins were constant throughout development. The Con A-binding glycoproteins, which are localized to the external surface of the postsynaptic membrane overlying the PSD, are present in similar quantities in immature (4-16 days) and adult SJ fractions. However, large differences in the composition of Con A-binding glycoproteins were observed in SP fractions during postnatal development. An exception to these developmental similarities was the major FSD protein which, when compared to adult SJ, was present in SJ and SP fractions at approximately the same levels throughout the period of active synaptogenesis (8-18 days postnatal).

These results show that post synaptic membrane glycoproteins were present in Con A-Sepharose 4B columns 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 basic adhesion. In contrast, the major FSD 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.)
SOCIETY FOR NEUROSCIENCE


Sucking 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 discernable 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 onogenesis of the caudate nucleus. If the lead exposure, however, is related to a 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)


In the chick embryo, the combined use of electrophysiological and retrograde labelling techniques have shown that appropriate neural projection patterns, involving a tight correlation between axon termination site and motoneuron soma position, are established prior to muscle cleavage and the 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 lumbarosacral spinal segments L5-3 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 lumbarosacral 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 were not innervated by 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.

The second series of experiments consisted of the reversal of segments L5-3 at the same stages. Preliminary results from spinl nerve stimulation at Stage 35 revealed that the displaced motoneurons had established connections with the 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.)

351 ALTERNATING RETINAL GANGLION CELL TERMINATION BANDS IN DOUBLY INNERRATED PROX OPTIC TECTA. Margaret L. Law* and Martha Constantine-Paton. Dept. Biol., Princeton University, Princeton, NJ 08540.

Two optic tracts were induced to innervate a naive tectal lobule through the implantation of a third eye prismaordium into the forebrain region of Shumay stage 17 Rana pipiens embryos. Autoradiographic analysis following intracocular injections of 3H 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 lobule.

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 compositely excluding axons from the alternate eye.

Electrophysiological recordings reveal that both the normal and the supernumerary 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 lobule 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.)

*Weisel et al., Brain Res. 79: 273, 1974; Graybiel, Brain Res. 96: 1, 1975; Nobile et al., Brain Res. 96: 25, 1975; Shatz et al., Brain Res. 131: 103, 1977.
SEGMENTAL SELECTIVELY INNERVATED OF MAMMALIAN SYMPATHETIC GANGLIA: RELATIVE ROLES OF INTRAGANGLIONIC POSITION AND POST­GANGLIONIC TARGETS. Jeffrey W. Lichtman, Joseph W. Ying 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 related, either directly or indirectly, to its intraganglionic position or some aspect of its postganglionic target.

By analogy with other systems, selectivity might reflect a topographic matching of sympathetic ganglionic neurons with different spinal segments to particular regions of sympathetic ganglia. The influence of intraganglionic cell position in the superior cervical ganglion, however, was very great. Neurons were classified by their position along the major ganglionic axes receive, on average, the same segmental innervation. Moreover, horseradish peroxidase-labeled cells on 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. We used the test activation of sympathetic targets within a particular region of the body.

To determine whether the pattern of segmental innervation is more closely related to the position of its soma within the ganglion, however, cannot be very great since neurons located at different positions along the major ganglionic axes receive, on average, the same segmental innervation. Hence, horseradish peroxidase-labeled cells on 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. We used the test activation of sympathetic targets within a particular region of the body.

The isoforms of the glycylcysteine enzyme, enolase (E.C.4.2.1.1) from rat brain have been extensively characterized 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 enolase activity (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 isoform was studied in six brain areas of newborn rat brain up to 25 days of age. In all six regions, brainstem, cerebellum, quadrangular plate, hypothalamus, forebrain and olfactory bulb, NSE levels are low at birth, (1 ug/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 precursor neurons that differentiate or acquire 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 Res. Res. 19:213, 1977) it appears that the neuronal isoform is replaced by the glial isoform as the neuronal antigen is replaced by the glial antigen, and that NSE synthesis is repressed, and the gene for NSE derepressed in the course of neuronal differentiation. The appearance of NSE at a late point in the development of the nervous system and functional maturation is in its final stages suggests that the neuronal enolase has properties specifically suited to the physiology of the functional neuron.

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. Vaughan. 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 pyramidal cells (JCH 156: 417, 1974), provide an opportunity to study development and acquisition of functional 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 SJL/J mice. This reversal was limited to the portion of regio inferior that displays genetically-associated variations in the connectivity pattern of the mossy fiber system, as revealed by the Timm sulfide silver method. In both strains, the formation of the majority of the granule cells occurred in development occurs in development occurs in a linear day 19 and postnatal day 7 in both strains. In addition, the percentage of the total labeled granule cell population that was labeled on the injection day was significantly different in different 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 injection occurring at 5-7 days. Other regions are more delayed with cerebellum being the slowest. The late appearance of NSE in the brainstem suggests that the neuronal antigen 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 Res. Res. 19:213, 1977) it appears that the neuronal isoform is replaced by the glial isoform as the neuronal antigen is replaced by the glial antigen, and that NSE synthesis is repressed, and the gene for NSE derepressed in the course of neuronal differentiation. The appearance of NSE at a late point in the development of the nervous system and functional maturation is in its final stages suggests that the neuronal enolase has properties specifically suited to the physiology of the functional neuron.


The production of acetylcholine (ACh), y-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 the lamina (first synaptic neuropil), with that in a more proximal portion containing the medulla and lobula complex (second- and third-order neuropil) and the distal portion of the optic lobe, the labeling of the pools of neurotransmitter candidates was linear with time. At the conclusion of an incubation, the ratio of radioactivity in the distal portion of the optic lobe to that in the proximal portion is about 1:5 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 animals lacking an eye on the operated side. Light microscopy of the optic lobe is 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 ACh, GABA, and 5HT in the proximal and distal portions of the optic lobe, we examined the binding of [125I]a-bungarotoxin to frozen sections in autoradiography (in collaboration with Dr. L. Hall, MIT). The distribution of ACh and GABA in silver-stained sections of the medulla were similar in operated and control lobes. When the production of ACh, GABA, and 5HT was examined in the distal portion of the optic lobe, it was found that ACh labeling was 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 the optic lobe of normal unoperated animals. These findings will be discussed with reference to the neurotransmitter chemistry and development of the cellular components of the optic lobe in Manduca sexta.

This research was supported by an NIH Postdoctoral Fellowship to GDM and NSF Grant BNS 77-13281 to JGH.


The MFB is located in the ventrolateral diencephalon and project to the basal forebrain and via its massive axon terminal connections with the limbic forebrain and midbrain forms the major longitudinal conduction tract of the hypothalamus. Intersitial neurons of the MFB (lateral preoptic and hypostriatum ventrale) are the reticular-type and are interpersed within a complex and heterogeneous fiber system. Path neurons of the MFB have fusiform, poorly myelinated, small diameter axons that are restricted to a single generation pattern, and the formation of granule cells was detected unequivocally on postnatal day 6 and the densely-stained mossy fibers were already visible throughout regio inferior. The prominent appearance of the MFB in neonates prior to the acquisition of functional activity correlates with the only major change thus far identified in a developmental event that might produce the connectivity differences seen in adult rats.

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 the injection day was significantly different in different 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 injection occurring at 5-7 days. Other regions are more delayed with cerebellum being the slowest. The late appearance of NSE in the brainstem suggests that the neuronal antigen 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 Res. Res. 19:213, 1977) it appears that the neuronal isoform is replaced by the glial isoform as the neuronal antigen is replaced by the glial antigen, and that NSE synthesis is repressed, and the gene for NSE derepressed in the course of neuronal differentiation. The appearance of NSE at a late point in the development of the nervous system and functional maturation is in its final stages suggests that the neuronal enolase has properties specifically suited to the physiology of the functional neuron.

No differences have been found between these strains either in the development of the granule cells, as examined by thymidine autoradiography, or in the temporal appearance of the mossy fiber system, as revealed by the Timm sulfide silver method. In both strains, the formation of the majority of the granule cells occurs in development occurs in a linear day 19 and postnatal day 7 in both strains. In addition, the percentage of the total labeled granule cell population that was labeled on the injection day was significantly different in different 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 injection occurring at 5-7 days. Other regions are more delayed with cerebellum being the slowest. The late appearance of NSE in the brainstem suggests that the neuronal antigen 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 Res. Res. 19:213, 1977) it appears that the neuronal isoform is replaced by the glial isoform as the neuronal antigen is replaced by the glial antigen, and that NSE synthesis is repressed, and the gene for NSE derepressed in the course of neuronal differentiation. The appearance of NSE at a late point in the development of the nervous system and functional maturation is in its final stages suggests that the neuronal enolase has properties specifically suited to the physiology of the functional neuron.

Investigations of FC have shown age-related changes in gross morphology as well as neuronal cell loss. Removal of FC in aged rats produced a significant reduction in the number of units in the solitary complex (nucleus and tractus solitarius) of the aged rats, 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 technique.

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 intermitotically not only among fibers in a bundle but also between the fibers and the somas and processes of migratory cells. Such intimate contact may 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 mitochondria. The fiber bundles are typically oriented parallel to one another and are connected via direct intercellular bridges identical to those described in the developing cerebellum by Ibs (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 ovoid vesicles separating two cells. No other membrane structures or 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 (3-5/2). 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 undivided state. No such bridges have been observed among Phase II cells as yet.

Although the temporal occurrence of these junctions during the entire migratory process has not yet been determined, the above observations suggest a communicative role important to some phase of migration much like that proposed by Loewenstein (Devel. Biol. Suppl. 2: 151, 1968).

Supported in part by NIMH grant MH-27677.

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 were transplanted orthotopically to replace corresponding hindbrain levels of chick embryo hosts. In the second series, neural crest and adjacent neural folds of caudal hindbrain levels 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 DE02458-03.


During normal development there is a decrease in the number of motor neurons in the lumbar spinal cord of amphibians (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 nerve growth factor (Pittman, R.H., Oppenheim, R.W. Nature 1971:214). 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.


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 (measured by a terminal photocell beam) 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 50% at 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, rates were injected with 100 mcg/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, onogenic changes in modification by preliminary stimuli were the same for experimental and control groups. Over days 14 to 16, 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 reduction in acoustic startle response 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 dopamine in the control of developmental processes. Experiments are now in progress examining the relationship between changes in neuronal uptake of 3H-NB produced by 6-OHDA and the behaviors just described.

(Supported by PHS grant NS-12443.)

This work was supported by the SNF (3.432.74)

The literature has described 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 form was thought to be a "marker" for nerve-muscle contact (Vigny et al., J. Neurochem. 27: 1347, 1976) and is approximately 185 in eel electroluminescence. In rat and 19.5S in chick, 15.5S was not detected in primary cultures of chick muscle (Rotund 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.

Our findings confirm and extend earlier observations of the sensitivity of the EMI 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-01736.)


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% Pb 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 neurones dorsalis 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 on the molecular layer of the visual cortex of 5½ ft. using a tethering device and recorded according to the Scholl method. Fifty neurones 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 on the molecular layer of the visual cortex of 5½ ft. using a tethering device and recorded according to the Scholl method. Fifty neurones 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 on the molecular layer of the visual cortex of 5½ ft. using a tethering device and recorded according to the Scholl method. Fifty neurones 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%.

Our findings confirm and extend earlier observations of the sensitivity of the EMI 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-01736.)


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 experiments were conducted to evaluate the role played by the periphery in the development of the neuronal circuitry responsible for generation of the stereotyped, bilaterally synchronized motor pattern of wing-flapping. Flapping of wingless chicks was made visible by mounting sections of nodal straws on the stumps of the amputated wings. Flapping was evoked by dropping chicks a distance of 5/4 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.

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.

This research was supported by Grant 840292 from the National Research Council of Canada.


The literature has described 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 form was thought to be a "marker" for nerve-muscle contact (Vigny et al., J. Neurochem. 27: 1347, 1976) and is approximately 185 in eel electroluminescence, 165 in rat and 19.5S in chick, 15.5S was not detected in primary cultures of chick muscle (Rotund 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.

Our findings confirm and extend earlier observations of the sensitivity of the EMI 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-01736.)

COMPARISON OF TRANSNEURONAL CHANGES IN THE AVIAN ECTOMAMILLARY AND VENTRAL LATERAL GENICULATE NUCLEI. J. D. Peduzzi*, E. S. Baran* 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 recessed 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 (experimental 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 (35%) 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 EMI 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-01736.)
SEGMENTALLY SELECTIVE INNERVATION OF MAMMALIAN SYMPATHETIC GANGLIA: FURTHER EVIDENCE FOR THE IMPORTANCE OF POSTGANGLIONIC TARGET POSITION. Dale Purves, Jeff W. Lichtman, and Joseph W. Vip. 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 neurons whose axons run to two postganglionic nerves, the inferior and superior branches. Since the branches of the superior nerve innervate more rostral caudal spinal nerves than those of the inferior nerve, the apparent difference in innervation of particular superior cervical ganglion cells is not primarily determined by the position of targets along the rostral caudal axis. However, the position of targets may affect the segmental innervation of neurons running in the divisions of the inferior nerve to the second and third cervical nerves:

- 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 in the ventrolateral neck, C3 in the cervical and dorsal regions. The apparent influence of dorsoventral position on segmental innervation was supported by recording sympathetic compound action potentials from the cervical nerves in response to stimulation of the thoracic ventral roots. Those branches running to dorsal regions showed a stronger response to stimulation of relatively rostral ventral roots than branches running ventrolaterally to the cervical plexus.

On the basis of the experiments reported in these abstracts, we conclude: (1) Sympathetic ganglion cells connect with the position of their postganglionic targets. Thus segmentally selective innervation of sympathetic ganglia appears to match positional values of pre- and postganglionic neurons. (Supported by NIH grant NS-11694.)


Cerebrovascular permeability to 14C-sucrose and other water-soluble nonelectrolytes normally is equal to nonelectrolyte permeability in aporous, bimolecular lipid membranes (Ohno et al., Am. J. Physiol., in press). It has been suggested that cerebrovascular permeability with an equal brain-permeability-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. (32) to measure cerebrovascular permeability to 14C-sucrose in 3-month and 28-month old, conscious Fisher 344 male rats. 5 µCi 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, Obrain, was obtained by subtracting intravascular tracer (product of blood concentration and brain blood volume) from net regional brain blood activity. It was assumed that tracer entered brain as follows (1):

$$d[14C_\text{brain}] / dt = P_{\text{plasma-brain}} [14C_\text{plasma}] - [14C_\text{brain}].$$

where $P_{\text{plasma-brain}}$ is the cerebrovascular permeability-area product (sec$^{-1}$), $V_c$ is cerebral distribution space of 14C-sucrose (ml/g), $t$ is time, $O_{\text{brain}}$ = brain tracer concentration (dpm/g) and $O_{\text{plasma}}$ = plasma concentration (dpm/ml). Plasma concentration was represented by the following equation (2):

$$O_{\text{plasma}} = A e^{-k_1 t} + A e^{-k_2 t} + \ldots,$$

where $k_1$ and $k_2$ are the cerebral blood flow and the plasma clearance rate of 14C-sucrose, respectively.

Eq. 3 was fit by computer to concentration data to obtain PA and $V_c$. PA in 3-month-old rats averaged 7.53 £ 10$^{-3}$ sec$^{-1}$ in 14 brain regions and was not significantly different in 3-month and 8-month old rats, except possibly at white matter and then by not more than 30%. Furthermore, we fell from an average of 0.126 in young animals to 0.068 £ 10$^{-3}$ in 28-month old rats. This decrease probably resulted from 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.

This phenomenon is unlike the generalized coupling and uncoupling that occurs during postnatal cerebrovascular development (Potter, Furshpan & Lennox, PNAS 55:328, 1966). Since R2 and P1 develop in separate ganglia, the two axons are presumably initially coupled but then become functionally uncoupled during a period of about one week. Until stage 11, action potentials in each cell are 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 0.5. A loss of one-for-one firing occurs at the transition between stages 11 and 12. In parallel, the coupling factor decreases to about 0.2 for 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 one-way biventricular PF in P1 following an R2 spike, characteristic of the adult (Menier & Tauc, Arch. Ital. Biol., 111:305, 1973).

This phenomenon is unlike the generalized coupling and uncoupling that occurs during postnatal cerebrovascular development (Potter, Furshpan & Lennox, PNAS 55:328, 1966). Since R2 and P1 develop in separate ganglia, the two axons are presumably initially coupled but then become functionally uncoupled during a period of about one week. Until stage 11, action potentials in each cell are 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 0.5. A loss of one-for-one firing occurs at the transition between stages 11 and 12. In parallel, the coupling factor decreases to about 0.2 for 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 one-way biventricular PF in P1 following an R2 spike, characteristic of the adult (Menier & Tauc, Arch. Ital. Biol., 111:305, 1973).
376 INFLUENCE OF UNDERNUTRITION ON THE DEVELOPMENT OF THE SLEEP - WAKE CYCLE OF RATS. José A. Rojas-Amat‡ and Ana Ma. Martínez-Rivas*. Fac. Medicina, UNAM, México 20, D.F. México. Since relationships between protein synthesis and sleep mecha-

377 MATURATION OF COCHLEAR POTENTIALS IN THE RAT, AND THE EFFECT OF KANAMYCIN ON THE ONSET OF THE AUDITORY FUNC-

378 LIFESPAN CHANGES IN THE DIURNAL SLEEP PATTERN OF RATS. Richard S. Rosenzweig and Allen Rechcigl, Sleep Laboratory, University of Chicago, Chicago Ill. 60637. TABLE: Changes in rhythm amplitude measures with age. 

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 in seven discrete brain regions which are known to have varying vulnerability and functions. 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.
DECREASE IN ADRENERGIC AXON SPROUTING IN THE SENESCENT RAT.

much lesser extent than that in young adult animals. This may
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)

The location of motoneurons innervating extensor and flexor
muscles in the leg and thigh was investigated in normal and extra
limbs. 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, Neurons injected in the septum and hippocampus of aged (28-31
month old) and young (3-6 month old) Sprague-Dawley rats. Axon
sprouting of adrenergic neurons innervating the rat septal and
hippocampal areas was studied following a unilateral transection
of the fimbria using a modified glyoxylic acid histofluorescence
method for the cellular localization of monoamines. Both young
and aged animals were subjected to transection 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 innerva-
tion in response to denervation. Thirty to sixty days after fimbria transaction 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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 trans-
sections induce the growth of sympathetic catecholamine neurons
to the septum and hippocampus (Spencer & Cotman, 1976). The effects of
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 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 such behaviors can be readily elucidating the neurochemical bases of motor behaviors since the ontogeny of such behaviors can be readily elucidated. We have focused our attention in this regard on elucidating the neurochemical bases of motor behaviors 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 such behaviors in rats of the same age given 20mg/kg of the muscarinic blocker atropine every 8 hours for 7 days. The forebrain and 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, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors exhibited by the normal animals, such as a decline to the lower, adult frequencies.
TIME OF ORIGIN OF NEURONS IN NUCLEUS CUNEATUS LATERALIS IN THE MOUSE. Elizabeth Taber Pierce, Seri Slaad* and Inger J. Onshus*, Dept. Anat., Harvard Med. Sch., Boston, MA 02115

Female mice Balb c/cm were mated to SJL males. The pregnant mice were injected with tritiated thymidine, 5 µg/ml body weight. A series of off-spring was obtained pulse labeled with tritiated 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 µ 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.


Pregnant female rats of the Long-Evans Hooded strain were injected on days 13-20 of gestation (spontaneous vaginal smear day 0) with diazepam (2.5, 5, or 10 mg/kg) or the vehicle. Marked sedation of the dam was noticeable at the highest dose of diazepam (5). 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 diazepam injected rats (7) delivered on day 21. Hence, prenatal ds 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 ds-exposed pups became increasingly apparent as the ds-exposed pups did not sustain the same growth rate as the control groups. By 104 days conceptual age the postnatally 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 at each peak at 38 days conceptual (16 postnatal) age and declining sharply thereafter. Animals exposed to the lowest dose of ds 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 ds, 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 ds-exposed animals. Evaluation of the startle response against increasing background intensities demonstrated further effects of prenatal ds exposure. All control groups showed potentiation at a background intensity of 75 db whereas no potentiation was observed in any of the ds-exposed pups. These studies have demonstrated that exposure of the fetus to ds during the period of marked neuronal differentiation can have pronounced and long lasting consequences.

EXPRESSION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES DURING DEVELOPMENT OF THE AUTONOMIC NERVOUS SYSTEM. G. Teitelman*, H. Baker, T.H. Joh, and D.J. Reis. Laboratory of Neurobiology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

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-ß-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT), whether sympathetic and adrenergic (suprarenal) neuroblasts express TH and DBH simultaneously during development, that a transient expression of PNMT is required for the expression of PMNT and that a transient population of noradrenergic cells is present in the developing, growing sympathetic ganglia prior to the expression of PNMT. We propose that migrating sympathoblasts express TH and DBH simultaneously during development, that PNMT expression is transient, that CA synthesizing enzymes are 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 ganglia and b) the adrenal medulla. Support: National Institute of Child Health and Development, National Institutes of Health.
AUTORADIOGRAPHIC LOCALIZATION OF \(^{3}H\)-ESTRADIOL IN RELATION TO NEURONAL ACTIVITY AND CYCLIC NUCLEOTIDE LEVELS ARE IN PROGRESS IN AN ATTEMPT TO ELUCIDATE THE INTRACELLULAR MECHANISMS CONTROLLING THIS DEVELOPMENTAL TRANSITION.

Recent evidence strongly suggests that individual sympathetic neurons, when taken from the cervical ganglia in 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 exogenous cyclic nucleotide derivatives on this choice of transmitter were studied. Adrenergic and cholinergic development and functional properties of the labeled cells were similar to that of the neonatal rat (Sheridan et al., Endocrinology, 94, 1386, 1974) and of the adult mouse (Warenbourg, C.R. Acad. Sci., 250, 152, 1970; Stumpf and Sar, Anatomical Neuralendocrinology (Karger) 82, 1975). The effects of several Ca agonists and antagonists were studied. Neurons exposed to 1 mM butyrate did not mimic these effects. Agents which elevate the effects of depolarization were studied. Neurons exposed to 1 mM butyrate did not mimic these effects. Agents which elevate calcium in astrocytes include EGTA, or D600) largely blocked the effect of K+ on cholinergic neurons and the pattern and regional localization of the steroid nucleus of the stria terminalis; arcuate and ventral preamillary areas. A. Dominique Toran-Allerand, John L. Gerlach*, and Bruce S. McEwen. Columbia Univ., ColI. PAS, New York, N.Y. 10032, and Rockefeller Univ., New York, N.Y., 10021.

Cyclic AMP-dependent protein kinase (PK) and protein phosphorylation in human brain tissue postmortem. 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 rats reared 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 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?

We raised kittens in the dark for 40 days and then divided them into 3 groups: (1) active locomotion, (2) passive exposure, (3) raised in the dark until tested. Groups 1 and 2 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 death was delayed until after active locomotion in the light; none could see the limbs or locomote.

Preliminary results indicate that passive exposure to an environment provokes a change in the orientation of the kitten toward the visual cliff.
AGE-RELATED CHANGES IN SPINE NUMBERS ON CEREBELLAR BASKET CELLS IN POST-NATAL RATS. Christopher D. West (SPHN: Michael J. Maloney). 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, silivered, embedded in low-viscosity-nitrocellu­ lous 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 branches den­ drites 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 Pur­ kinje cell dendritic spines revealed no corresponding decrease. (Supported by USPHS Grant No. 1 R23 AG06677-01 from the NIA and by the Veterans Administration).

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 re­ sponses in individual ganglion cells. Such recordings show a segmentally selective pattern of innervation. Although each neuron in the mature ganglion typically receives synaptic con­ tacts 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 pre­ ganglionic axons to a neuron provides the dominant innervation to that cell (as measured by either the amplitude of the post- synaptic 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 segment. 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 T7 and T5, 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 inner­ vation 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 terri­ tory they innervate. [Supported by NIH grant NS-11699.]


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 Rambberger- 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- bulge) 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 (Jaheri 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.)
DRUGS OF ABUSE
After injection of ethanol, there was a significant decrease in the activity of the enzyme that catalyzes the synthesis of cytoplasmic brain proteins following chronic exposure to dietary alcohol. We suggest that the decreased synthesis of cytoplasmic brain proteins following ethanol withdrawal may be responsible for the behavioral changes observed in offspring — derived from male parents injected with morphine, but not from saline-derived controls. In the second study, both male and female offspring — derived from male parents injected with morphine or saline — were tested at 12 weeks of age on a step-through avoidance procedure (1.6 mA scrambled foot shock, 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.

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.

The neuronal intranuclear body (INB) of the hamster brain, first reported by LaVelle and LaVelle (Exp. Neurol., 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 perfused 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 where 46% of control cells contained a prominent INB vs 2.7% of cells exposed to alcohol. Measurements of the prominent INBs in both control and alcohol treated animals showed a statistically significant increase (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. In these experiments, the size of the largest INBs the mean diameter increased from 1.67 µm (control) 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.

Studies were conducted on 27 adult rats, locally anesthetized, and paralyzed with tubocurarine and artificialy respired. Recording semi-microelectrodes were implanted in the caudate, amygdala, and at two levels of the caudal grey (CG) and projecting (SN) structures. EEG and unit activity were recorded in the olfactory bulb and substantia nigra (SN). Stimuli of 4 intensities were delivered as 5-sec trains of 10/sec pulses. Activity changes were filtered and averaged. Diurnal differences 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 its frequency in all areas except the amygdala. Naloxone caused a naloxone-reversible increase in response time in the amygdala of many rats and a decrease in others. Morphine's effect on SN responses was significantly depressed by prior morphine treatment. The effects of opiates on single unit responses of the hippocampus were studied and compared to those previously obtained in medial thalamus. The effects of NMDA on hippocampal units were studied and compared to those previously recorded in medial thalamus. Morphine's main effects on hippocampal EEG were a morphine-induced increase in response time and a decrease in response rate. The majority of hippocampal responses were increases in response time. The effects of morphine on hippocampal unit responses were not different in terms of absolute latency, or latencies relative to cortical EEG changes from those previously recorded in medial thalamus. There was a marked difference in the pattern of responses in the two areas. Whereas medial thalamic units had 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 change 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 N, the majority of responses of hippocampal cells following an injection of 0.0125 mg/kg naloxone in drug-naive, chronically-prepared, paralyzed rats. 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. There was a marked difference in the pattern of responses in the two areas. Whereas medial thalamic units had 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 change 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 N, the majority of responses of hippocampal units following an injection of 0.0125 mg/kg naloxone in drug-dependent animals (1-75 mg pellet implanted s.c. for 3 days) were sustained decreases in rate. Hippocampal units were less responsive to this low dose of naloxone (also 0.0125 mg/kg) 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 changes in the cortical EEG.

These results constitute evidence that medial thalamic units are more responsive to the drug than are hippocampal units. It is possible that the latter are more susceptible to the effects of the drug, or that they are less sensitive to the drug, or that they are less sensitive to the effects of the drug. It is possible that the latter units are more susceptible to the effects of the drug than are hippocampal units. It is possible that the latter units are more susceptible to the effects of the drug than are hippocampal units. It is possible that the latter units are more susceptible to the effects of the drug than are hippocampal units.
407 INTERACTION BETWEEN MORPHINE AND REWARDING OR EUPHORIGENIC PROPERTIES. 

MORPHINE ON BRAIN STIMULATION REWARD. N. A. Loens and S. M. Sallati*. 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 septal area (LSA) and medial prefrontal cortex (MRF). The animals were run in 10 min sessions at least once daily. A 5 min period separated testing at each electrode site. Different doses were injected after determining for a threshold current intensity that had stabilized (see Psychopharmacol. 48 (1976) 217). Different drugs and doses of morphine and naloxone (NOX) were tested after saline 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 mg/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 chloridiazepoxide (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 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 5,6-dibromo-3-furylmethyl-3,4-dihydroxy-6,7-benomorphan (5.0 mg/kg) blocks the facilitatory effect of ETOH (0.4 g/kg) and CDP (4.0 mg/kg) on LH SS. M2266 at 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 endogenously-opiate peptide 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.


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 a surprising finding that morphine (M) administered i.v. to albino rabbits produces only a transient miosis which is followed by larval light sensitivity and an increase in pupil size. 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 was able to record pupil size changes at 40 sec intervals. No other treatments were given after saline 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 mg/kg) or the other compounds. The animals then were tested hourly for 5 hr post-injection.

Systemic injections of morphine increase the total amplitude of saccades and reduces the duration of fixation, indicating a decrease in the time required to process information as indicated by decreases in 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 ICSS was replicated and compared with the ICSS effects with and without morphine in the septal area (LSA), periaqueductal grey (PAG) and the mesencephalic reticular formation (MRF). The results indicate marked stimulation site dependent differential morphine effects, as well as inter-subject consistency. ANOVA test groups differed markedly from non-stimulated controls. The LSA subjects, compared to the LHA subjects, showed moderate rates of shutting and a similar increase in total ON time, decrease in shutting and increase in average ON time when given morphine. The PAG subjects showed immediate averse to the ICSS, which had baselines characterised by low rates of shutting and very low ON time. These two subjects under morphine generally further decreased ON time and rates of shutting. 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 ICSS stimulation related to an aversion suppression hypothesis of morphine action.
413 8-ENDORPHIN-INDUCED ALTERNATIONS 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, MSS 1A8.

Interactions between brain monoamines and the effects of opiates have been described, 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 intracerebral administration of synthetic human 8-endorphin (15 µg) on DA and 5HT metabolism in discrete brain regions were investigated in adult male Sprague-Dawley rats. 8-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 concentrations of DOPAC and HVA were greater in endorphin-treated than in saline-treated animals, supporting the concept that 8-endorphin increases striatal dopamine turnover. 8-endorphin also increased the rate of decline in striatal DA following synthesis inhibition with 8-methyl-tyrosine, further suggesting that endorphin increases striatal DA turnover. It is probable that this apparent increase in striatal DA turnover is compensatory since 8-endorphin appears to inhibit neuronal release of DA. 8-endorphin and probenecid interacted competitively to decrease the effects of each other to increase striatal HVA, andnaloxone prevented this effect of 8-endorphin.

In addition to the effects on striatal DA metabolism, 8-endorphin increased concentrations of 5HT and its metabolites, 5-hydroxyindoleacetic acid (5-HIAA) in brain stem and hypothalamus and decreased 5-HIAA in hippocampus. 8-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, 8-endorphin appears to increase 5HT turnover and release in brain stem and hypothalamus and decrease 5HT turnover and release in hippocampus, while at decreasing 5HT reuptake in these brain regions or increasing 5-HIAA egress from brain.

Acute administration of 8-endorphin clearly alters brain DA and 5HT metabolism. The relationship of these changes in brain monoamine metabolism with 8-endorphin-induced alterations in behaviour, pain threshold, thermoregulation, etc., remain to be defined.

Supported by MRC DA-58 and MRC MA-5183.

414 THE EFFECT OF INTRAVENOUS ETHANOL ON VOLUNTARY ALCOHOL CONSUMPTION BY ALCOHOL-PREFERRING RATS. Marshall B. Waller, William J. McTavish, Lawrence Jutwet* and Ming-Yi Jan (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 metabolism.
EPILEPSY
418 LESIONS OF THE INTERPEDUNCULAR NUCLEUS RETARD DEVELOPMENT OF AMYGDALOID-KINDED SEIZURES IN RATS. Robert F. Ackermann, and Jerome Engel, Jr., Neuropsychiatry Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Effects of ventral tegmental lesions on electrical amygdaloïd 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.

Following 2 weeks of daily handling, each animal was stimulated once a day with 60 cps, 400 μA current for 1 sec. until 3 consecutive stage 5 seizures were induced. Subsequently, the focus of the stimulating electrodes, and the focus 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=5.0) and the lesion controls (x=5.0), therefore they were combined into a single control group. Animals lesioned in the interpeduncular nucleus (IPN) required a significantly greater number of stimulations to induce 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 Tad, 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 amygdaloïd kindled seizures is retarded by IPN ablation, suggesting that the IPN normally facilitates kindled epileptogenesis. At present, a mechanism for this facilitation is not apparent, 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, M. Arnold, L. Renaud, Division of Neuropsychiatry, Montreal 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 associated with the increases in levels of GABA seen in many areas of the brain including the cerebral cortex. However, the precise mechanism of action of the site 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 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 microprobes were fixed to 7-barrel microiontophoresis electrodes filled with L-glutamate, GABA, acetylcholine (ACh), bicuculline, and sodium valproate (5.0, pH 8.0). 40 neurons displayed enhanced excitability of rapid onset and termination during ejection of sodium valproate and bicuculline from a 1 mm needle near the cortical surface. Application of L-glutamate also showed enhanced excitability of rapid onset with all 40 of these neurons. The effects of sodium valproate were reversible, although only a small "priming" current was necessary. The sodium valproate and L-glutamate enhanced neuronal excitability in a non-saturable manner. ACh also increased the excitability of over half the neurons tested. Sodium valproate increased the excitability of both cells sensitive and non-responsive 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 and 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)


Subpalial injections of 5 μl of 100 mM FeCl₂, FeC₁₅₀₄ or 0.9 NaCl were instilled into the sensorimotor cortex of Sprague-Dawley rats. Regular cortical electroencephalographic recordings through extra- durally implanted scalp electrodes revealed the following: 1) Both ionic salts of iron caused focal spiking activity within 48 hours, with spread of action potential foci to the sensory and parietal cortices and dorsal hippocampus in 10 days; 2) Behavioral convulsions and electocorticographic 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 spiking 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 fully lesionated region 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. Simonson* and C.L. Smith, Vanderbilt University School of Medicine, Carbondale, Ill. 62901.

In an attempt to localize the seizure antagonizing effects of morphine (M), seizure susceptibility was examined in rats after systematic 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 (1.5mm diameter) into the brain at the level of the inferior colliculus according to the procedure described by Ernoff et al. (Proc. Soc. Exp. Biol. Med. 150, 748, 1975). Biochemical assessment of the lesion by 25-35 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 pentylentetrazol seizure test was found to be significantly decreased (p<0.01) by the lesion. However, no difference in the threshold for maximal electroshock seizure was detected between sham-operated and lesioned animals. Inasmuch as seizure facilitation has been consistently observed following widespread destruction of NE neurons, the present results cannot be attributed to the lesion-induced reduction in forebrain NE. It seems more likely that damage to structures other than the NA neurons is responsible 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.

The term "kindling" describes the phenomenon whereby repeated focal electrical stimulation induces electrophysiological and behavioral changes consisting of increasing duration and spread of neural afterdischarge (AD) activity and increasingly 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 microtubophosphorhesis of glutamic acid (GA):0.02M pH4.5, gamma-aminobutyric 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 biphasic square waves, 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 no growth of ACH subsensitivity. A subsequent kindling stimulus delivered during 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 with 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 total of 110 male hooded rats were implanted bilaterally with bipolar twisted nichrome electrodes and stimulated between 100 and 200 times each through an 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 a 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.


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 (Ii) presumably carried by Ca2+. We have postulated that Ii is an important factor in penicillin-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, Ii is necessary for penicillin-induced bursting since no bursting is observed when Ii disappears due to injury produced by electrode impalement. Thus, we have hypothesized that Ii can dominate neuronal firing behavior if the repolarizing potassium and leak membrane currents (I1, and I4, respectively) are decreased or if I1 is increased. Specifically, a relatively small decrease in potassium e.g., 80mV (E), caused by the raised extracellular potassium accompanying intensive neuronal activity may reduce I1 sufficiently to allow Ii to produce bursting. To test this hypothesis we have adopted the expedient of decreasing E, (and, thus, I4) by intracellular injection of the relatively impermeant cation tetramethylammonium (TMA). This agent appears simply to displace intracellular Ca2+ 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 I4, and a small decrease in I1, allowed Ii to become net inward over a certain range of membrane potential. The interspike plateau duration 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 E4 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, (and, thus, I4). It is concluded that a relatively small reduction of E, in the presence of a normal I1 is sufficient to cause bursting. This may be one mechanism by which normal neurons are recruited into seizure activity. (Supported by VA Research Grant NRIS 1610.)

KINDLING IN SENSORY THALAMUS AND NEOCORTEX. A PRELIMINARY STUDY. Donald Peter Cohn and Marilyn Willbrodt*. Dept. of Psychology, U. of Western Ontario, London, Ontario N5A 5C2 Canada

The kindling response of a number of sensory and motor areas of thalamus (lateral geniculate, medial geniculate, lateral posterior, and posterior nuclear), 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 each through an electrode at the individually determined ACH supersensitivity. 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 no growth of ACH subsensitivity. A subsequent kindling stimulus delivered during 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 with 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.


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 focal epilepsy (SAS). We have demonstrated that a 9-year-old boy developed partial seizure activity characterized by formed auditory perceptions ( voice, 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 BA. 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 BA. 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.

A number of recent studies using a variety of model neural systems have suggested that penicillin (PCN) greatly reduces 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 IPSPs elicited by bicuculline, a GABA-antagonists' neurons by stimulation of the anterior vermal cerebellar cortex.

Ten cats were anesthetized with pentobarbital (6) or a-chlordiazepoxide (4). The cortical EEG was continuously monitored to detect spontaneous interictal or seizures. It was specifically considered the possibility of a progressive decrease in IPSP amplitude in parallel with the development of abnormal EEG activity as predicted from the above hypothesis. Intracellular recordings with KCl filled microelectrodes were obtained from 10 Deiters' neurons. Penicillin doses (2x10^6 IU/kg) sufficient to cause frequent interictal spiking or seizures were associated with spontaneous high frequency volleys of Deiters' neurons, "depolarization shifts," and large, spontaneous IPSPs. We found that in normal cells the IPSP may decrease over time concomitantly 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 those 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 within a single or even within a series of PCN. In particular, there was no consistent IPSP decrement as EOG or Deiters' cell activity progressively increased. Analysis of IPSPs in populations of cells before and after PCN in 9 experiments also suggested that PCN caused no significant change in IPSP amplitude in the cell populations. These results can be doubted on the basis that reduced inhibition due to PCN may alter GABA antagonism is necessary to cause seizures. It is possible that cortical GABA receptors are more sensitive to PCN than their mammalian counterparts. We cannot in the present study can be extended to the brain slices maintained in vitro. (Supported by VA Research Grant NR 1610 and NSC Grant NS05006.)

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 unrestrained expression of excitatory synaptic events. The epileptogenic effects of benzyl penicillin have been found to reduce presynaptic inhibition in the spinal cord (Davidoff, Br. J. Exp. Pathol., 1972) and certain postsynaptic inhibitions in invertebrates (Hillman & Kung, 1974; Hocner et al., 1975). 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. Intra cellular 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 antidromic stimulation yielded a mixed EPSP-IPSP, while stronger stimulation evoked a single action potential. Penicillin, applied as a small drop (20 ml 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 antidromic 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 receptors are iontophoretically affected. Concurrent with the gradual reduction of the recurrent IPSP by penicillin, the mixed IPSP-EPSP elicited by subthreshold antidromic stimulation was converted to a greater proportion of a 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, 1978), and is in accord with their finding that the presynaptic fiber volley and field EPSP are unchanged. The present study was supported by VA Research Grant NR 1610 and NSC Grant NS05006.


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 100 ppm are about 53, 38, 22, and 0. Rats were exposed to either 1000ppm or 600ppm CO for 2 hr and injected with either 30, 40 or 50 mg/kg pentylmenetrazol. Again 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 5 sec after a 15 minute resting period. 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 electrical stimulation (AD) properties were studied. CO hypoxia had no effect on the size or the parameters of the rebound AD. Reduced AD's occurred in 100% of the O2, 80% of the 250ppm, 60% of the 500ppm and 0% of the 1000ppm groups. The significance of the rebound AD's and their disappearance under hypoxia is not known. It may be pointed out that reduced AD's decrease in frequency with dosages of sodium pentobarbital as low as 10mg/kg. However, the AD model is the most reliable of those tested for studies of CO-induced hypoxia.
CHANGES IN RETICULAR FORMATION NEURONAL RESPONSES TO SENSORY STIMULI INDUCED BY STRYCHINE. C. L. FAINGOLD and J. D. STITTSWORTH, Jr., Division of Pharmacology, Dept. of 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 areas and occurs at a lower dose of strychnine than that which enhances the responses in other non-primary sensory sites (Faingold, Neuropharmac., 16:73-81, 1977). This study was undertaken to explore the neuronal events associated with this response enhancement in the RF utilizing locally anesthetized, paralyzed rats. 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. In contrast, previous reports suggest that these convulsants induce only minimal changes in sensory-evoked field potentials (Faingold, Neuropharmac., 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.)

METOCLOPRAMIDE [PMZ] THRESHOLD IN RATS WITH LESIONS OF THE SUBSTANTIA NIGRA (SN). R. G. FALCETTO and O. HONGNES. Dept. of Neurology, University of Wisconsin, Madison, Wis. USA and Clare Institute of Psychiatry, Toronto, Canada

Three groups of preselected rats with a stable PMZ threshold (3 generalized convulsions (fear test score above 10 in three tests in different days) for the tests) were used for the study. Group A was injected with 20 mg/100 g body weight saline in the left SN. Group B received 1.0 mg/kg 6-hydroxydopamine (6-OHDA) and group C was sham operated. As we have previously reported (Shibuya et. al., Exp. Neurol., 58, 484-499, 1978) such lesions induce changes in DA and metabolite content in the ipsilateral substantia nigra. The time course and the magnitude of these changes are illustrated in fig. 1 where the continuous line refers to group B and the dotted line to group A. Rats of the three groups were tested for PMZ threshold at various times after surgery. At the end of the experiments they were sacrificed and histologically examined and 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. Electrocorticography (ECG) showed an increase in threshold. ECG showed an increase in threshold and the evoked response of cobalt on extra-striatal structures showed changes in the opposite direction to DA changes in the neostriatum. Group C did not show variations. Cobalt caused a progressive neocortical lesion extending over time beyond the SN whereas electrophysiology induced a small stable lesion in the SN. Therefore it seems that the continuous decay of the PMZ threshold in group A is unrelated to DA changes and linked to the epileptogenic action of cobalt on extrastriatal structures. These data suggest that the endogenous opiate systems do not mediate the seizure phenomena of this model of epilepsy. (Supported in part by National Institute of Health.)


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 they may play a role in seizure phenomena. Opiate antagonists have been shown to have anticonvulsant activity and to protect the adult gerbil from seizures.

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, 20 mg/kg) and 4) acute naloxone treatment in conjunction with phenobarbital (20 mg/kg) administration.

In a) 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.

In b, c, and d acute treatments, no changes were noted in the seizures of the animals treated with the opiate antagonists.
NEURONAL INTERACTIONS IN EXPERIMENTAL EPILEPTOGENIC PROCESSES. Richard N. Harner, Otto M. Eggert*, 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 S1 during 1-2 msec 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 heterogeneous 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.

OPERANT CONDITIONING OF 12 - 16 Hz SENSORIMOTOR RHYTHM REDUCES MOTOR SEIZURES MORE THAN PSYCHOMOTOR SEIZURES. William J. Jackson, Arden V. Nelson*, and June G. Kearns*. Department of Neurology and Biomedical Engineering, Medical College of Georgia, Augusta, GA. 30901.

In our previously intractable psychomotor epileptics were compared to a group of previously intractable epileptics with focal motor or generalized convulsive disorders following 75 operand training sessions. Patients had been unable 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 only the patients realized improvement in their seizure condition as a result. Improvement was greater in the group with focal motor and generalized convulsions. With all of these patients showing what appeared to be 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 number of seizures during training. An additional factor involved the design of the devices which analyzed the signal prior to feeding a cue back to the patient. 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 elliptic 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 in 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. N01-NS-6-2346)

EPILEPTIC FOWL. D.D. Johnson, J.L. Davis*, and R.D. Crawford*. Saskatchewan, Sask. Canada S7N 0W0

The seizure process in epileptic fowl is sensitive to the anticonvulsant activities of phenobarbital (PB), phenytoin (Ph) and primidone (Pr) but not to ethoxzemide. 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 Ph 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 atropine was reduced in 1, 3, 6, 12 and 24 hour 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

ANTICONVULSANT ACTIVITY OF DIPROPYLAETIC ACID (DPA) IN EPILEPTIC FOWL. D.D. Johnson, B.I. Davis* and R.D. Crawford*. Depts. of Pharmacol. and Animal & Poultry Science, Univ of Sask. Saskatchewan, Sask. Canada S7N 0W0

The seizure process in epileptic fowl is sensitive to the anticonvulsant activities of phenobarbital (PB), phenytoin (Ph) and primidone (Pr) but not to ethoxzemide. 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 Ph 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 atropine was reduced in 1, 3, 6, 12 and 24 hour 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


Trimethadione (TM) 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 TM on synaptic transmission between identified neurons in the abdominal ganglion of Aplysia californica.

Simultaneous intracellular recordings were made from the cholinergic interneuron LiO and two monosynaptically connected follower neurons, L5 and R16, which receive an ipsps and epps, respectively. We found that TM (1-10 mM) reduced the amplitudes of both epps's as well as LiO'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 TM on both LiO's action potential and its followers epps's. This observation, coupled with an earlier finding that TM increases "interneuron II" activity (Kreisman et al. Comp. Biochem. Physiol., in press), implies that the effects of TM on synaptic transmission from LiO may be the consequence of actions at other sites.

In order to avoid the problem of recruited synaptic input and to test TM's effects on presynaptic mechanisms, we examined the cholinergic eppp produced in LiO by stimulation of the right connective. A 1 sec train of stimuli to the right connective produced an initial depression of eppp size followed by facilitation and posttetanic potentiation (PTP) (Schlapeke et al. Brain Res. 76:267-280, 1974). We found that TM (1-10 mM) produced a dose-dependent decrease in the size of all epps's of a train without affecting the relative degree of depression, facilitation and PTP. Simulation of "interneuron II" input to LiO altered the eppp, but only the eppp of an initial depression of "interneuron II". Bath application of acetylcholine (ACH) was conducted in the absence and presence of TM to test for a possible post-synaptic site of action. TM had no effect on the depolarization produced in LiO by ACh.

These results are consistent with a presynaptic action of TM leading to reduction transmitter release. The mechanism of the reduction in transmitter output will be the subject of further investigation. (Supported by grants from NIH-NS-12149 and the Schneider Foundation.)

143
CORTICO-CAUDATO-THALAMIC INTERACTIONS IN EXPERIMENTAL FOCAL EPILEPSY. John A. Rauske, Div. of Neurological Surg., UCI-Institute, and VA Medical Center, Los Angeles.

To study the interaction of corticofugal, cortico-caduatothalamic networks during the propagation of seizures neuronal activity was 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 tunicatic 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 somatotopically related to the cortical focus; a fourth electrode was placed in the ipsilateral ventral anterior thalamic nucleus. 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. Receiving seizures, with propagation of afterdischarge to all recording sites, changes 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 somatotopically 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 or focal neuronal activity it is apparent, here at least, that thalamic activation must occur before seizure afterdischarge can be recorded in the caudate nucleus.

TABLE I

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Cortex</th>
<th>IC</th>
<th>C (non-IC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tau</td>
<td>2.25(0.45)</td>
<td>1.94(0.07)</td>
<td>5.60(1.05)</td>
</tr>
<tr>
<td>Asp</td>
<td>3.49(0.80)</td>
<td>2.94(0.94)</td>
<td>3.56(1.59)</td>
</tr>
<tr>
<td>Gin</td>
<td>2.35(0.55)</td>
<td>1.99(0.32)</td>
<td>2.46(0.58)</td>
</tr>
<tr>
<td>Glu</td>
<td>6.36(1.41)</td>
<td>5.33(1.56)</td>
<td>11.32(3.90)</td>
</tr>
<tr>
<td>Gly</td>
<td>1.57(0.44)</td>
<td>1.33(0.47)</td>
<td>0.70(0.29)</td>
</tr>
<tr>
<td>GABA</td>
<td>2.94(1.41)</td>
<td>2.75(1.33)</td>
<td>1.50(0.65)</td>
</tr>
<tr>
<td>OTHER amino acids</td>
<td>2.50(0.38)</td>
<td>2.20(0.34)</td>
<td>3.56(0.31)</td>
</tr>
<tr>
<td>TOTAL amino acids</td>
<td>21.46(1.83)</td>
<td>18.48(1.55)</td>
<td>28.70(3.38)</td>
</tr>
</tbody>
</table>

Values represent means ± S.D.

The injection of 2 μg of the potent excitatory neurotoxin, kainic acid (KA) (Coyle and Schwarzw, 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 (DSP), hyperactivity (HA) and generalized convulsions (CNV). The effects of various anesthetics and anticonvulsants on the behavioral and neurotoxic actions of a threo-bose 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 displayed 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 91%. Anesthesia with PB alone (65 mg/kg) provided some protection while CH alone (400 mg/kg) was ineffective against KA. Treatment of ether-anaesthetised animals with the anticonvulsants diazepam (10 mg/kg), phenoobarbital (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 unique model for studies on the mechanism of sedation, if it can be shown that KA fails to induce LTP in the dentate gyrus.

Supported by NIH Grant NS 04053 and NS 09677.


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 hemispherotomized temporal lobe patients have been reported (Simpel, et al., Epilepsia, 15, 1974) and putative changes in hippocampal structures in kindled rats are presently under investigation in our laboratory. For the purpose of investigating these areas, Golgi studies were performed at various stages during development in the CD50 strain. The present study is directed at determining whether either intracerebral recordings can be obtained from human epileptic foci in patients undergoing cranotomy and cortical resection (Calvin, Ojemann & Ward, Exp. Neurol. 46:583, 1975).

Outcome: a) permanent disruption of the blood-brain barrier;
b) endocrine dysfunction; c) impairment of GABA synthesis;
d) neurodevelopmental abnormalities; e) lowered metabolic efficiency.

Increased Seizure Susceptibility in Adult Mice Following Neonatal Treatment With Glutamate or Aspartate. William J. Pizzi, June H. Barnhart* and James R. Unnertall*. Neuropsychology Lab, Northwestern Illinois Univ., Chicago, IL 60625

Neonatal administration of the amino acids glutamate (GLU) and aspartate (ASP) has been shown to be anticonvulsant in a variety of mammals. This report presents data demonstrating that neonatal administration of GLU and ASP can produce adult mice that show an increased susceptibility to the neurotoxicity of a potent agent (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 males.

Neonatal mice were given subcutaneous doses of GLU, ASP or base saline on days 2 to 11 after birth and were subsequently exposed 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% of the species (C57BL) 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 CD50 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 also showed a tendency to become obese (equated weights). A third group of GLU-treated mice showed increased susceptibility to PTZ (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 and a treatment was found that must 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). A fourth group of GLU-treated mice were given freshly prepared aspartic acid. These animals showed a tendency to become obese (equated weights). All probability values are two-tailed.

Increased Seizure Susceptibility in Adult Mice Following Neonatal Treatment With Glutamate or Aspartate. William J. Pizzi, June H. Barnhart* and James R. Unnertall*. Neuropsychology Lab, Northwestern Illinois Univ., Chicago, IL 60625

Neonatal administration of the amino acids glutamate (GLU) and aspartate (ASP) has been shown to be anticonvulsant in a variety of mammals. This report presents data demonstrating that neonatal administration of GLU and ASP can produce adult mice that show an increased susceptibility to the neurotoxicity of a potent agent (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 males.

Neonatal mice were given subcutaneous doses of GLU, ASP or base saline on days 2 to 11 after birth and were subsequently exposed 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% of the species (C57BL) 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 CD50 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 also showed a tendency to become obese (equated weights). A third group of GLU-treated mice showed increased susceptibility to PTZ (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 and a treatment was found that must 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). A fourth group of GLU-treated mice were given freshly prepared aspartic acid. These animals showed a tendency to become obese (equated weights). All probability values are two-tailed.

Increased Seizure Susceptibility in Adult Mice Following Neonatal Treatment With Glutamate or Aspartate. William J. Pizzi, June H. Barnhart* and James R. Unnertall*. Neuropsychology Lab, Northwestern Illinois Univ., Chicago, IL 60625

Neonatal administration of the amino acids glutamate (GLU) and aspartate (ASP) has been shown to be anticonvulsant in a variety of mammals. This report presents data demonstrating that neonatal administration of GLU and ASP can produce adult mice that show an increased susceptibility to the neurotoxicity of a potent agent (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 males.

Neonatal mice were given subcutaneous doses of GLU, ASP or base saline on days 2 to 11 after birth and were subsequently exposed 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% of the species (C57BL) 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 CD50 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 also showed a tendency to become obese (equated weights). A third group of GLU-treated mice showed increased susceptibility to PTZ (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 and a treatment was found that must 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). A fourth group of GLU-treated mice were given freshly prepared aspartic acid. These animals showed a tendency to become obese (equated weights). All probability values are two-tailed.
CONVULSANT ACTION OF HYPOGLYCEMIA: CORTICAL DISINHIBITION. W. Reabe. Dept. Neur., 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 neural 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 (IHI) and i.v. ammonium-acetate (AA) on cortical postsynaptic inhibition and cerebral ammonia-concentration were studied. Pentobarbital anesthetized and artificially respirated rats were used. Extracellular recordings were obtained from pyramidal tract cells. Cortical postsynaptic inhibition was measured as the efficacy of current 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.

IHI (100 U.i. 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 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 currently 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.

ALTERED PHOSPHORYLATION OF CORTICAL MEMBRANE PROTEINS AFTER ECS. N. M. Reddy, E. V. Bittor, L. C. Davis, J. Daugherty & E. C. 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 CMP in various brain areas. Frequent alterations in the laboratory have indicated that such changes are accompanied by alterations in the phosphorylation of specific membrane-bound proteins, presumably of synaptic origin (Braun, R. et al.). In the present study, the effects of electroconvulsive shock (ECS) on this enzymatic activity have been investigated. Rats (120-140 gm) were sacrificed by body immersion in liquid N2. Portions of the brain were dissected and placed in a chilled, buffered (pH 7.4) medium. After removal of the meninges the membranes were isolated by gentle homogenization and centrifugation. The membranes were prepared as described (Pharm. Biochem. Behav. 6: 169, 1979). Endogenous phosphorylation reactions were carried out by incubating the membranes for 10 sec with gamma-32P-ATP (Nuclotron, Res. 21: 533, 1977) and the reactions were stopped by solubilizing the membranes to a detergent (15 SDS). Incorporation of radioactive phosphate from ATP into specific protein components was determined by autoradiography of reaction products separated electrophoretically in gradient (7-16%) polyacrylamide gel-slab. Over twenty specific protein bands that incorporate radioactive phosphate under these assay conditions were identified. In a separate set of experiments, the phosphorylation of one group of bands, designated H (OM 15-20K) demonstrated temporal relationships with the treatment. Compared to the above controls, phosphorylation 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.
HISTOPATHOLOGY OF FERRIC-INDUCED EPILEPTOGENIC FOCI: A GOLGI SURVEY IN CATS. S.A. Reid*, W.H. Borga*, G.M. Sypryt and L.L. Williamson. (SPON: Domestic cats were rendered epileptic via sub-pial injection of 20 microliters of saturated ferric chloride (FeCl₃) 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 FeCl₃ was compared to the homotopic contralateral cortex. Changes noted in the FeCl₃ 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 may be an accurate model of human focal epilepsy. (Supported by VA Hospital Medical Research Service.)

EFFECT OF PHENYTOIN ON THE ACTION POTENTIAL OF A NEURON IN THE LAMPREY SPINAL CORD. Michael E. Selzer. Dept. Neurol., Sch. Med., University of California, San Diego, CA 92103. 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 micro-electrodes, 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 Ca²⁺ and addition of 1 mM Mn²⁺ 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 µM (N = 60) or in washout (N = 41). PTN did not significantly affect the average resting membrane potential (50.9 ± 1.0 mV) or the input resistance (21.9 ± 1.0 mho). It did reduce the maximum rate of rise of the spike (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 or reduces the conductance 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.

GABAergic AXON TERMINALS DECREASE AT EXPERIMENTAL SEIZURE FOCI IN RODENT CORDS: A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY. J. E. Vaughn and W. L. Cragg. (SPON: 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.) GABA is a biogenic amine neurotransmitter that is synthesized in the brain by the GABA synthesizing enzyme, GAD. GABAergic neurons are at least partially blocked by the GABA antagonist, bicuculline. GABAergic nerve terminals were found to be distributed homogeneously in all layers of normal rodent spinal cords. Using an immunohistochemical method for the localization of GABAergic nerve terminals, we observed that in the rat neocortex where GABAergic nerve terminals are found to be distributed homogeneously in all layers of normal rodent neocortex and formed extensive pericellular plexuses with the soma of pyramidal neurons in layers III and V, GABAergic nerve terminals in monkey neocortex also formed pericellular synapses with the soma and dendrites of pyramidal neurons, suggesting that they arise from the same type of interneuronal circuits. Spinal cord sections from contralateral homotopic cortex. Sections from these sites (A, B, and C) taken from each of the subjects were examined at the same magnification and the numbers of GABA-positive terminals were counted in 36 contiguous µm² areas from the bottom of layer VI to the middle of layer V. The mean numbers of GABA-positive terminals per µm² 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. A loss of GABAergic innervation at seizure foci could be responsible for the observed epileptic activity.

Supported by USPHS grants NS10220, NS04061, NS09037 and NS12116.

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 epilepsy (Pokorny et al, Neurosci. Lett. 3:145, 1975). The present study was undertaken to ascertain the time relation between the administration of an enantioselective 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, ataxia and myoclonic jerks with occasional aphonia 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. Finalised 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 a microbiological method. The GHB concentration 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, which changes had their origins in subcortical areas (Sneed et al, Neurology 26:51, 1976).
HIPOCCAMPAL AFTERDISCHARGES AND NITROUS OXIDE IN ALZHEIMER'S DISEASE.


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) produced by stimulation of the HPC of the rat, and shown how these properties are altered by exposure to toxicants. The present experiment was performed as 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 doses of SP upon the tctal 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, 30 or 40 mg/kg SP. The threshold AD was produced to provide a standard of comparison for testing the effect of SP. At 30 mg/kg there was an increase in threshold for producing an AD, a large drop in the number of AD's followed by a further decline in AD duration. At 30 mg/kg the threshold increased further and the spike rate during the AD declined. 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 ictal activity. At 30 mg/kg 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 Racine method followed by a depression at the 0 mg/kg dosage, and this incidence was reduced further to less than 15% 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.

ALTERNED PROTEIN SYNTHESIS WITHIN A RECURRENT SEIZURE FOCUS IN THE ALBINO RAT. L. J. Willmore, A. J. Dunn, L. J. effing, and G. W. Proctor. 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 discharges. Electroencephalographic recordings with transcranial stimulation show 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 meningeocerebral cistern, neuronal neurulation and astrogial 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 [H]lysine, 1 μCi/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 hemisphere, and from the cerebellum. Tissue processed for liquid scintillation counting showed increased total uptake of [H] in the actively discharging epileptic focus, but the relative incorporation of [H]lysine into protein was decreased. Animals not developing epileptiform discharge showed diminished uptake of [H] without alteration in relative incorporation of [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. The decrease 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.)


Afterdischarge (AD) and postictal depression (PID) resulting from seizures in the hippocampal formation (HPC) depend upon the properties of the eliciting stimulus. Stimulation at 400% of threshold produced short afterdischarges compared to those elicited by stimulation at 115% of threshold. It is not yet known to what extent the PID of the HPC is an accurate reflection of depression both excitation and depression. If PID duration accurately reflects depressed excitability then high intensity stimulation should be followed by a longer period of excitability 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 excitation than high intensity stimulation. The purpose of the present work was to determine the excitability of the HPC after an AD and its relationship to AD and PID duration. Male hooded rats were implanted with 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, 30 or 40 mg/kg SP. The threshold AD was produced to provide a standard of comparison for testing the effect of SP. At 30 mg/kg there was an increase in threshold for producing an AD, a large drop in the number of AD's followed by a further decline in AD duration. At 30 mg/kg the threshold increased further and the spike rate during the AD declined. 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 ictal activity. At 30 mg/kg 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 Racine method were followed by a depression at the 0 mg/kg dosage, and this incidence was reduced further to less than 15% 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.

MEDIAL FOREBRAIN BUNDLE LESIONS PROLONG AMYGDALAR AFTERDISCHARGE WITHOUT CHANGING THE ONSET OF BEHAVIORAL SEIZURES IN THE KINDLED RAT. MC. Waller and L. C. Crawford. Southeastern 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 when the threshold for the eliciting stimulus is lowered and is electrophysiologically responsive, yet it has not been determined whether kindling is dependent on specific pathways and neuromodulators or if the effect is a global change in the brain. Stimulation at 400% of threshold produced short afterdischarges compared to non-lesioned control animals (28.8 ± 3.2 s). The data supports the concept that monoamines influence the stability of kindled amygdaloid seizures by making electrocorticographic lesions in the posterior medial forebrain bundle (MFB). Male hooded rats weighing 165-185 gm 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 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 (± 0.3 days, SDM) in the non-lesioned rats. A similar number of days (7 ± 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 non-lesioned 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 = 4) on the lesioned side (756 ± 158 ng/mg wt. wt.) compared to the contralateral side (754 ± 610 ng/mg wt. wt.). Norepinephrine and serotonin levels were reduced 25% to 40% and 40% to 60%, respectively in tissues from the lesioned side. The averaged decrease in dopamine charge was not significantly different from zero for rats with MFB lesions (49.5 ± 4.5s) than for non-lesioned control animals (28.8 ± 3.2s). The data supports the concept that monoamine neurotransmitters influence the duration of afterdischarges. The 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. The lesions may dissociate electrophysiologic events from kindled seizure behavior. (Supported by a grant from the Epilepsy Foundation of America and the VA Medical Research Service).
EVOKED POTENTIALS AND EEG
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=13) 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 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 components for the reflective subjects. In addition, the reflective 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.

1This research was supported in part by a Grant from the Office of Naval Research, Physiology Branch.

IDENTIFICATION OF VOLUME-CO Conducted EARLY EVOKED POTENTIALS IN THE SOMATOSENSORY SYSTEM OF CAT. Philip C. Bechter* and Robert J. Schlabass. 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 neuroaxis 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 neuroaxis. 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-T32-00747.)
Two recursive and two a posteriori Wiener filters were applied to each evoked potential from the somatic responses to stimulation of the frontal, central, or posterior parietal regions. The outputs of the filters were then compared with the original evoked potentials to determine the effectiveness of the filtering procedure. It was found that the recursive filters were more effective in removing noise than the a posteriori filters, but that the a posteriori filters were more effective in enhancing the signal-to-noise ratio. The results of this study suggest that the Wiener filtering method may be useful in improving the quality of evoked potentials for the purposes of clinical diagnosis and research.
Supported in part by USPHS Grants NS10471 and RRO5755. Band intensity, may reasonably be associated with use of an event-related spectral intensity, accompanied by decreased alpha and beta bands. 

Supported in part by DARPA through ONR contract N000-14-76-C-0002. Block structures (BR), serial addition of a column of decimal digits (AR), substitution of letters with subsequent word recognition (KB), mental paper folding (FO), fixation on a spot (EO), and recovery cycles, will help in assessing the functional significance of the cortex and brainstem to ischemia was also made with 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 recovery of the evoked potentials following impact in animals receiving mild injuries. The appearance of coma following 1.7 - 2.0 ATM injuries correlated with cortical dysfunction as the brainstem evoked potentials remained unchanged from baseline. 

**Table I** 

<table>
<thead>
<tr>
<th>Average % Performance on Independent Validation Data</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EO</td>
<td>WR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>93</td>
<td>95</td>
</tr>
<tr>
<td>99</td>
<td>95</td>
<td>97</td>
</tr>
<tr>
<td>98</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>99</td>
<td>96</td>
<td>95</td>
</tr>
</tbody>
</table>

Supported in part by NIH grant NS 12587 and a Southern Medical Association Training Grant.
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-trifluoroethyl difluoromethane) 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) temperatures. Enflurane produced latency shifts (slowing) in positive peaks II through V(p<0.001), with the largest changes occurring in later waves. In addition, enflurane reduced the amplitude of waves PIII, PIv and PV relative to PI (p<0.001). In curarized animals, reducing mean rectal temperature by 2°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 (e.g., caffeine). These potential influences on 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.


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 influence BAER latencies indirectly. As evidence of this, we have shown that the P1 (1100 Hz) 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 different rates of maturation have been presumed to reflect separate peripheral and central processes (EEG cclin. Neurophysiol. 42:418, 1976). The tone-evoked auditory brainstem responses, another indicator of central neural plasticity, were used in this investigation 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 is mediated along the spinal cord to the brainstem. This method may be used to assess whether low spinal injury spares ventrolateral columns.

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. Peter). Brain-Behavior Research Center and Letterman Army Medical Center, Sonoma State University, Eldridge, CA 95431

Developmental studies of the brainstem auditory click-evoked response (BSEER) in monkeys have shown that the P1 (1100 Hz) 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 different rates of maturation have been presumed to reflect separate peripheral and central processes (EEG cclin. Neurophysiol. 42:418, 1976). The tone-evoked auditory brainstem responses, another indicator of central neural plasticity, were used in this investigation 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 is mediated along the spinal cord to the brainstem. This method may be used to assess whether low spinal injury spares ventrolateral columns.
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 preference (i.e. Life Sci. 18: 693-702, 1976). The present study was designed to compare bi-laterally recorded secondary components of the visual evoked potentials (VEP) of rats with lesions in the nigrostriatal system and Metrazol-induced wave-spike afterdischarges with rotational side preference as assessed in the rotometer. Both naive undrugged rats displayed reliable interhemispheric asymmetry of the secondary components of the visual evoked potentials. 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 (3% solution) injections which transformed secondary afterdischarges into wave-spike complexes. With the convulsive dose of Metrazol the symmetry of wave-spike afterdischarges was observed during the recovery phase after a generalized electrographic fit. Administration of amphetamine on the background of the Metrazol effect revealed a rotational behavior in the direction away from the hemisphere with more suppressed wave-spike activity. A decrease of the synchronized and hyper-synchronized electrocorticographic wave-spike activity over the hemisphere opposite to the preferred rotational side is believed to reflect the higher DA content in the more active hemisphere. 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 cortical surface in response to a visual stimulusalways followed by food reinforcement (Pharmacol. Res. Commun. 9: 669, 1977; Pharmacol. Biochem. Behav. 6: 697, 1977). The present experiments were designed to investigate whether amphetamine alters evoked potentials in slow potentials (SPs) associated with operant performance. Two seconds after the onset of a 20 msec auditory warning signal, a rat was placed on a lever. 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 1 sec after the rat left the lever at the end of the 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 the cortex with the brain being kept warm 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 referred reference electrode was located 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- and orientation will be specific to 1) those gratings with frequency, as compared to same orientation, as the attended grating (feature-specific attention), or 2) only that grating identical to the attended grating (pattern-specific attention). 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 in each of three instances 18-70 sec). Amphetamine (0.25 to 1.0 mg) caused a dose-related depression of SP amplitudes and areas in A-P and anterior S-D recordings. SP amplitudes of posterior S-D SPs were slightly depressed. However, the area 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-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 the 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.)

LINGUISTIC PERFORMANCE IN THE CONTEXT OF LANGUAGE NEUROPHYSIOLOGY. Armando F. Rocha* and E. Francozo* (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 and presentation. In Portuguese, as in many other languages, renders Given and New information in a sentence. New is the information the speaker (S) expects the hearer (H) to have in the mind at the moment of communication. Given and 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 Given and New is related to the intonational pattern H imposes on the test, and ilia set of 25 sentences, in which IE varied according to a different sentences and different intonational patterns of the same sentence, in order to reveal the patterns S imposes on the sentence. 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 and in sentences, the underlined words evoked easily recognizable EEG changes associated with 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 measures were enhanced when the stimulus was greater when the flashed grating had the same spatial frequency, as compared to same orientation, as the attended grating. The results were related to sensation processing and neurophysiological models of spatial vision. The mechanisms underlying selective attention to four features of attention depended on which point in time after stimulus the VEP amplitude was measured. Between 175 and 225 msec post-stimulus, the facilitary effect occurred when the flashed grating had either feature in common with the attended grating (feature-specific attention). After 225 msec post-stimulus, the facilitary effect occurred primarily when the flashed grating had both features in common with the attended grating—i.e., 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 measures were enhanced when the stimulus was greater when the flashed grating had the same spatial frequency, as compared to same orientation, as the attended grating. The results were related to sensation processing and neurophysiological models of spatial vision.

We have reported a number of evoked potential differences between psychiatric and nonpsychiatric populations in these studies were derived from discharge diagnoses arrived at by two senior psychiatrists. The question examined was whether differences in the patterns, which were 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), RSI clicks (AEP), or checkerboard pattern flash (VEP). Recordings were obtained from 14 scalp and one occipital lead.

The BPRS ratings of 150 hospitalized psychiatric patients were subjected to principal component factor analysis with varimax rotation. Individual scores comprised 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 symptomatology 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 finding was that 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 nonsympathetic 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 290-300 msec period over the nasal scalp. The scalp differences 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 nonsympathetic subgroup and the appearance similar to those of controls. Consequently, the results appear to be in accord with our previous reports comparing nonpatient controls and psychiatric populations.


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 (Exp. 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 (Exp. Neurol. 58:111, 1978). An average of 400 BEPs was summed 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 lyophilized for lipid analysis. The remaining hemispheres from three or four rats from each group were used for myelin and synaptosomes prepared using discontinuous sucrose gradient procedure. Results of these experiments showed that the rate of myelinosis becomes faster with 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 while 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 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 in 15 days. Since the amount of 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. The lipid 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.

By NIH Grants NS11670 and NS12424.

POW SPECTRAL DENSITY METHODS FOR THE DETECTION OF WEAK EVOKED POTENTIALS. Bernard Saltzeim, Information Analysis Section, Texas Research Institute of Dental 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/or latency instability 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 cases, however, the signal to noise ratio is high enough to allow detection of evoked potentials with spectral analysis. The waveform of the stimulus is modeled by spectral densities derived from the stimulus plus an additive noise. The power spectrum of each evoked potential is subtracted from this model and the residual spectrum plotted on a dB scale. A criterion for significant deviation of the residual spectrum from white noise is established. The coherence of the residual spectrum is then computed and any significant coherence seen is associated with a peak in the residual power spectrum. The relative position of this peak is used to estimate the latency of the evoked potential.

The significance of these methods in a re-evaluation of current theories on the SMR will be discussed.

In support of this work, the authors would like to acknowledge the contributions of E. A. Kaczmarek and C. J. Kelly.

Supported in part by a doctoral dissertation research fellowship from the Bollman and G. L. Modlin Research Fund of the Children's Hospital of Philadelphia and by Grant NS 2426 from the NINDS.

480 SINGLE-TRIAL AND SMALL-N VERTEX BRAIN POTENTIALS RELATED TO SENSORY THRESHOLD AND RELATIVE DURATION ESTIMATE FOR TWO MODALITIES IN HUMANS. Hilton Stowell. Sensory Neurophysiol., Rivers Lab, CSH, CA 91062.


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/0.2 msec) and recorded from VPL nucleus (mean latency of 18 msec) showed no significant changes during various states of wakefulness and sleep.

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 showed large variations according to the state of wakefulness and sleep. It was high during distractive 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 showed large variations according to the state of wakefulness and sleep.
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 asymmetries 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 (GAT1000). 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 in between was undertaken to minimize fatigue. The subsequent runs consisted of the subject voicing a mono-syllabic 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 larynx 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
**EXTRAOCULAR MOVEMENT**

**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 neuromotor system. Targets were presented as blue-green oscilloscope dots (100 x 100 pixels) thresholds which stepped horizontally to one of 6 or 8 randomly selected positions in the range 15 degrees. The subject's left eye was monitored in the dark by a special infrared tracking device, the right eye being occluded by an eyepatch. Seven subjects were asked to perform two tasks - to follow the target with the eye (normal saccade N) or to stop on an equal and opposite eye movement (anti-saccade A) - and feedback of latency and error was given automatically (Hallett, Vision Res. 15, 233). "Hallet" responses to the target (direct errors) occurred in only 5% of A trials. The mean A latency was typically prolonged and had a larger standard deviation than the N. Fraction times D or A, or differences such as A-D, vary considerably from subject to subject. However, the regression line of A on N is simply A = 2.01N - 143, with r^2 = .98. This implies that the definition of the goal at some fixed time G in the overlap region.


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 scans his visual environment. Time-lapse optic flow 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 latency of a saccade varies by more than 15 degrees predominate. Some of these smaller eye movements take the form of a glance where the intervening fixation may be neither longer than the latency for a saccade (N) or shorter than an equal one of opposite eye movement (anti-saccade A) - and feedback of this small and opposition eye movements or glances with such high frequency but the rate at which the larger saccades occur is the same as 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.

**FINE STRUCTURE OF SACCADE BURST UNITS IN THE MACAQUE BRAINSTEM.** J.A. Blair, B. Eckmiller and M. Gunston. 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. Neural 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 ms before each saccade, and stopped firing 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 1720 imp/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.

**CONNECTIONS OF A VERTICAL EYE MOVEMENT AREA IN THE ROSTRAL MESENCEPHALIC SEGMENTUM OF THE MONKEY.** J.A. Bütter-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 medial longitudinal fasciculus (MLF), which lies caudal to tractus retroflexus, but also the rostral interstitial nucleus of the medial longitudinal fasciculus (RMLF) and retrorolateral group (rostral retrolateral group) are 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 ([3H]proline and [3H]leucine) and retrograde (horseradish peroxidase) tracer substances in macaque monkeys.

HFP 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 that supply the subnuclei from the abducens nucleus, that is, the medial and inferior rectus divisions. The contralateral terminal labelling after an injection of [3H]proline into MLF and rostral iMLF was limited to the nucleus Darkschewitsch and rostral iMLF and rostral iMLF were shown to originate from fibers that crossed within the posterior commissure, and also included rostral iMLF, IC and its adjacent reticuliform formation. Control injections in n. subfasciculatus, the fields of Forel, the hypothalamus, the red nucleus and the n. Darkschewitsch labelled of none of the above structures. Long pathways which descends within the ipsilateral MLF were shown to originate from of the cuneiform and rostral iMLF. Bilateral cell groups receive vestibular afferents, although the pontine reticular formation, a center for vertical and horizontal eye movements, the smaller eye movements of less than 15 degrees.

The Society for Neuroscience, Mt. Sinai Sch. of Med., CUNY, New York, N.Y. 10029, and Univ. of Zurich, Zhi, 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 & Henn, 1978). OKAN has been shown to consist of two components: OKAN and per- and post-rotatory nystagmus in humans and monkeys and to determine the role of velocity storage in producing visually-vestibular interactions in man. The gain of OKAN in humans is 0.8-1.0 sec/90°. Similar gains were found for rotation in light, but for rotation in darkness vestibular gains varied from 0.15-0.75. Post-rotation in darkness and in light the monkey gains for rotation in dark and light are close to 1 to 1.80-240°/sec. During OKAN in the monkey eye velocity rises rapidly and then slowly reaches peak values. In humans eye velocity-rotation declines rapidly with 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 the intensity of after-nystagmus in both humans and monkeys. Consistent with saturation levels of OKAN, the visual input to OKN is weak and saturates at about 20/sec. The higher the OKAN in light, the lower the OKAN in darkness. The model indicates that direct pathways from OPN are excited by visual stimuli and it is not clear how this activity is related to enhancing the low frequency characteristics of the VOR than in supporting ocular following. It could also be important in mediating the presence of motion.

Supported by NINCDS Grant NS 00294 & Fellowship NS 05297(T.R.)

VELOCITY CODED NEURONS: A NEW CLASS OF PRE-MOTOR NEURONS IN THE PRIMATE OCULOMOTOR SYSTEM DURING PURSUIT. Raef Eckmiller, E. Handwerker.

Smith-Kettlewell Institute, San Francisco, CA 94115

Initially, Desning and Schaefer (Arch.Psychiat. Z. ges.Neurol.196:402,1957) working with rabbits, and then several authors working with monkeys described neurons in the pericentral cortex which were "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 12 monkeys exposed to a pursuit light spot (6 min.of arc in diameter), which was sinusoidally moving in the horizontal plane. EMs were recorded from 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 red nucleus nucleus increased with pursuit velocity to the right and vice versa for neurons located on the left side.

c) IR max during sinusoidal pursuit is 0.1 to 1.5 Hz in the on-direction typically did not exceed 100 impulses per second.

The maximum velocity stimulus VM in (in comparison, IR max of motoneurons during sinusoidal pursuit lagged 40 to 80 deg. behind VM 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.

ASYMMETRIES IN BINOCULAR COUNTERROLLING IN HUMANS DURING DYNAMIC ROTATION. Shirley G. Diamond*, Charles H. Markham, Norman E. Simpson* and Ian S. Curthoys*.


Seven subjects ranging in age from 18 to 66 years underwent 3 min. rotation about a naso-occipital axis at a constant velocity of 3°/sec in a specially constructed chair. Subjects were secured by a series of straps. The head was stabilized by means of a bite bar adjustable to permit precise horizontal alignment of both pupils. A 45x55 deg striped back-projected mirror 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 the measurement of pupil 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 countertrolling. 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 countertiroling at that position. This measurement was accurate to within one minute of arc: practical accuracy is between 15 and 30 minutes. Right and left eyes in each trial were measured independently.

Data from 7 subjects showed that OKAN and OPN in monkeys are weak and saturates at about 20/sec. Rotation in light is followed by a decrease in the intensity of after-nystagmus in both humans and monkeys. Consistent with saturation levels of OKAN, the visual input to OKN is weak and saturates at about 20/sec. The higher the OKAN in light, the lower the OKAN in darkness. The model indicates that direct pathways from OPN are excited by visual stimuli and it is not clear how this activity is related to enhancing the low frequency characteristics of the VOR than in supporting ocular following. It could also be important in mediating the presence of motion.

Supported by NINCDS Grant NS 00294 & Fellowship NS 05297(T.R.)
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 does not work, the visual world is seen as stationary, but the eyes move. If the gain of the VOR is gradually reduced, thereby improving visual stability. This adaptive plasticity of VOR gain has been shown in various species, including birds. These results suggest that birds can adapt to a reversed visual experience. Since at hatching chickens have typically normal vertical visual function, but only 0.24 in two one-day-old chicks. After two hours of rotation, the VOR gain was reduced to about 0.1. The time constant of this gain reduction was approximately one hour. These results are compatible with observations in primates.

In summary, vertical and horizontal oculomotor deficits as well as medial rectus motoneurones; and (2) vertical saccades; (2) deficits in vertical pursuit and in the control of the contralateral visual field. The effect of reversing prisms, the normal VOR destabilizes the visual world, resulting in an increased number of saccades. Because the power spectrums of the waveforms are flat from zero hertz to any predetermined frequency, the activity in the spinal nucleus of V. The VOR is gradually reduced, thereby improving visual stability. This adaptive plasticity of VOR gain has been shown in various species, including birds. These results suggest that birds can adapt to a reversed visual experience. Since at hatching chickens have typically normal vertical visual function, but only 0.24 in two one-day-old chicks. After two hours of rotation, the VOR gain was reduced to about 0.1. The time constant of this gain reduction was approximately one hour. These results are compatible with observations in primates.
The gain of neck controlled eye position during nystagmus. Neck proprioceptive stimuli alter the eye nystagmus caused by a unilateral injection of 0.5cc of 1% Lidocaine HC1 into the middle ear cavity of guinea pigs. Eye position was measured with Ag-AgCl ball electrodes serially implanted into holes in the left and right orbital crest that communicated with the orbits. Electrode signals were differentially amplified and displayed on a D.C. recorder. The eye nystagmus amplitude and direction were evaluated by comparing a videotaped record of eye position during nystagmus with concomitant EOG amplitude, or by measuring saccadic EOG amplitudes and frequencies induced by the horizontal voluntary head movements. Four parameters of nystagmus were measured as a function of maintained horizontal or vertical neck position: frequency of beat, 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 in 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 smaller left eye deviation. The effects were present in all 17 animals studied and were proportional to the amount of neck deviation imposed (maximum = 90° deviation). These influences, when compared to the situation with no neck deviation, when compared to the situation with no neck deviation, appeared regardless of the direction of the 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.

In conclusion, neck proprioceptive information influences nystagmus as a function of maintained horizontal or vertical neck position: frequency of beat, mean eye position, mean slow phase velocity, and direction of beating. The effects were present in all 17 animals studied and were proportional to the amount of neck deviation imposed. These influences appeared regardless of the direction of the 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.

Funded by a NASA grant to T.H. Bullock.
IS THE INTERSTITIAL NUCLEUS OF CAJAL A PREDOMINANT CENTER FOR VERTI­
NAL EYE MOVEMENTS? W.W. King, W. Precht* and R. Uhlrich-
* Max Planck Institute for Brain Res., Frankfurt/M, W. Ger.

The interstitial nucleus of Cajal (INC) may be a pre-motor cen­
ter 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 stud­
ies show that INC cells project monosynaptically to extracerebral
and neck 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 nemaline anesthetized cats
to determine the synaptic inputs to INC cells, and to test each cell
for antidromic activation from the cervical spinal cord. We were unable to demonstrate synaptic inputs from either
superior colliculus or optic chiasma.

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
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 EN07-22418 and NIH grant 1 R23
EY02382-01.

INTERACTION OF THE EYE MOVEMENT AND LOCOMOTORY CONTROL SYSTEMS.
Jose A. Laties*, A. Terry Babill, and B. Todd Troost. Neuro­
logical Control Systems Laboratory, Biomedical Engineering,
Carnegie Mellon University, Pittsburgh, PA 15213.

There have been many studies of eye movements and of lomoco­
try control, but none that focus on the interaction between the eye
movement system. Human locomotion is usually guided by in­
put from the visual system. To facilitate the information pro­
cess of this system, the eyes must remain oriented so the
fovea of the eyes are aimed at certain specific points. The head
oscillates up and down, from side to side, and from front to
back, while walking, but the horizontal plane platform for the
eyes, and compensatory eye movements must be made in order to
fixate the eyes on target while walking. Head turn of about one-half a degree, head sway of two or three degrees, and
compensating eye movements of ± five degrees were measured.

Inputs from at least three sensory systems are integrated to
guide the eyes movements. The visual system, the vestibular
system, and the proprioceptive system. Normal human eye
movements used while walking were studied with the feedback loop
closed on each system. A model of the human eye movement
control system was designed for while walking. It incorporates
the sampled data visual feedback aspects of the control system, the vestibulo-ocular
cortical formation, and the proprioceptive system, and the sixth
order nonlinear extraocular Reciprocal Innervation Plant (RIP).
A first step in investigating 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 simu­
late the plant so as to correlate the input-output relation of
real and the body RIP. The linear RIP model was justified qualitatively by comparing the shapes of the actual and
model movements, quantitatively by constructing Main Sequence diagrams plotting velocity versus magnitude and duration versus magnitude of eye movements for five conditions with the
same 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 EN07-22418 and NIH grant 1 R23
EY02382-01.

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 effect of OKAN, i.e. inducing more than 120°/sec, is not straightforward. Vertical OKN was induced by rotating animals 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 above 40°/sec. 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 OKO. The addition of a rotating gravity vector during pitch caused nystagmus to be prolonged indefinitely. This effect is similar to that seen during baroreceptor reflexes.

The presence of OKAN indicates that velocity storage occurs for nystagmus in the vertical as well as in the horizontal plane. Nystagmus (Okan) following rotation in liquid was weaker after rotation in darkness, indicating that velocity storage during OKN had reduced the post-rotatory response. Reduced storage for upward OKN was reflected in the ability to counteract post-rotatory downward nystagmus. Presumably during pitch the velocity storage integrator is continuously active and the rotation to the vertical in the labyrinth. The data suggest that the organization of the vertical and horizontal systems producing nystagmus is similar, but that there are in common a pool of neurons capable of velocity storage mechanism. Different coupling coefficients could also account for the observed differences between upward and downward nystagmus.

Supported by NIMH grant MH-51923

SOCIETY FOR NEUROSCIENCE


PGO waves are the prominent monophasic potentials seen in Pons, lateral Geniculate body (LGB) and Occipital cortex just before (transfocal) and after (transiently) the PGO burst. 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 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 - 60-90% 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 in midbrain sites in close approximation to the brachium conjunctivum, an area in which there is HRP evidence of projection from P8 to the LGB. The discharge pattern of these cells was 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 MH-51923
EFFECT OF PROLONGED OPTICAL REVERSAL OF VISION ON THE VESTIBULO-

velocity signals were significantly greater than head velocity sig­nals, since the signals carried by the flocculus changed less than the head velocity. Assessed were a measure of "head velocity" (vestibular?) sensitivity. Assessed was a measure of "eye velocity" sensitivity—and when examined during sinusoidal tracking both when the head was stationary--to provide a measure of "eye velocity" sensitivity. The discharges of horizontal gaze velocity neurons were examined for vestibular and oculomotor relationships: 44% were related to gaze velocity during horizontal tracking, 23% to vertical tracking, 15% to eye position, 4% to miscellaneous combinations of head and eye movement, 4% to saccadic eye movements only and 4% were unrespon­se. 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 moving exactly in concert with the target--to provide a measure of "head velocity" (vestibular?) sensitivity. We observed in this way in the non-normal monkey, head and eye velocity signals were of similar strength (correlation index = 0.87); regression line was not quite significantly different from zero and an intercept was not significantly different from zero. In the low gain animals, eye velocity signals were significantly greater than head velocity sig­nals (correlation index = -0.72; slope of regression line = -1.26; intercept not significantly different from zero). Such a shift in the weights of the head and eye velocity signals in low gain animals is consistent with the view that the flocculus is the site of modifiable elements suberving VOR gain changes. We suggest that such changes in the floccular output are more probably a secondary consequence of its 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.

VOR GAIN CHANGES PRODUCED BY TARGET ROTATION WITHOUT HEAD MOVE

Goldfish can be trained to change the gain of their vestibulo-
ocular reflex (VOR) over the course of a few hours (Neurosci Anim. 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 was rotated stationary coordinates. The VOR gain was measured by rotating the fish at the same period and amplitude in the dark. Fish were not rotated during the light period. In a typical experiment a fish underwent this "stripes only" treatment for times varying from two hours to six hours. In every fish the gain increased. The initial gain (EOG peak) was 0.61 ± .07, and the average gain was 0.99 ± .04. For comparison six fish that were trained for two hours in the stripes only condition increased their gain from 0.72 ± .08 to 0.95 ± .09. The peak amplitude of the VOR gain changes produced by target rotation without head movement in goldfish is significant compared to that seen in the stationary condition. 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 are blocked in pursuit related cells upon which floccular Purkinje cell axons terminate.

THALAMIC UNITS DETECTING ABSOLUTE SPACE POSITION OF VISUAL TAR

Saccadic 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 only in the contralateral visual field, if placed in the central visual field, should be oriented in the unit's preferred direction for producing saccadic bursts. These results, already published by several laboratories, suggest that the retina of the animal and the thalamus are involved in the control of eye movements. (Supported by NIH Grant EY01051)

PURKINJE CELL ACTIVITY IN THE MONKEY FLOCCULUS DURING SMOOTH PURSUIT EYE MOVEMENTS. Hirohara 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 anesthetized monkeys that were being trained to perform smooth pursuit eye movements in straight lines at various orientations on a tangent plane. Target movement was either as a sine wave or as a composite of sinusoids of different frequencies. The monkeys were trained in order to eliminate the predictability of a pure sine wave. Activity in Purkinje cells of the flocculus was studied in anesthetized monkeys that were being trained to perform smooth pursuit eye movements in straight lines at various orientations on a tangent plane. Target movement was either as a sine wave or as a composite of sinusoids of different frequencies. The monkeys were trained in order to eliminate the predictability of a pure sine wave. Activity in Purkinje cells of the flocculus was studied in anesthetized monkeys that were being trained to perform smooth pursuit eye movements in straight lines at various orientations on a tangent plane. Target movement was either as a sine wave or as a composite of sinusoids of different frequencies. The monkeys were trained in order to eliminate the predictability of a pure sine wave.
INUERATION 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 when, 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 intensively, 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. Non-peroxidase labelled cells could be labelled with impurities of the peroxidase injection. However, the sensitivity of the extrinsic ocular muscles means that we will be able to examine these projections. 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 NIH grants NS 12781, NS 00147 and NSF fellowship.)

1. 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 decreased and increased as the target moved away, the firing rate started to decrease. This was shown by a reduction in the ratio of the target velocity to the effective target velocity. The effective velocity is calculated in terms of the information needed for tracking than perception of movement.

2. In the present study, motoneurones innervating the four slips of the cat RB muscle have been identified by retrograde intraocular transport of horseradish peroxidase (HRP). Following injections of HRP into all four slips of the RB muscle, motoneurones were found distributed throughout the rostrocaudal extent of the ipsilateral abducens nucleus. The distribution of the LR and abducens inter-nuclear neurons was divided into four slips associated with the abducens nucleus. The RB motoneurones accounted for approximately 50% of the total number of neurons 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 oculo-motor nucleus, overlapping the distribution of MI motoneurones.

3. Injections of HRP into individual slips of the RB muscle also resulted in labelled motoneurones in the abducens, accessory abducens, and oculo-motor nuclei, though in fewer numbers than when all four slips were injected. There was no apparent topographical organization of neurons 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. In the rabbit, 3% - 5% of the total number of neurons in the abducens nucleus. Even at birth, the motoneurons of the rabbit oculomotor nucleus are segregated into distinct subpopulations, similar to those described in cat and monkey. These results directly implicate the floculus (and possibly para-floculus) in a variety of retinal image stabilizing reflexes. Smooth pursuit and cancellation of the 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 (352), 5) impaired suppression of the VOR when rotating with an optokinetic (OK) drum fixed to the chair (gain = 0.95; prop < 0.35), 6) abnormal OK nystagmus with a slow rise to maximum eye velocity (time constant; 15 secs; prop < 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, and eccentric gaze. These results directly implicate the floculus (and possibly para-floculus) in a variety of retinal image stabilizing reflexes. Smooth pursuit and cancellation of the 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 (352), 5) impaired suppression of the VOR when rotating with an optokinetic (OK) drum fixed to the chair (gain = 0.95; prop < 0.35), 6) abnormal OK nystagmus with a slow rise to maximum eye velocity (time constant; 15 secs; prop < 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.
FEEDING AND DRINKING
which a unilateral drug cannula aimed at the PVN was implanted. Tie stores; and third, bilateral parasagittal knife cuts between three hypothalamic manipulations known to increase feeding: first, the medial and lateral hypothalamus which cause hyperphagia and obesity. Rats received either large paramedian knife cuts rostro-lateral to the VM or sham knife cut surgery. Following recovery a second surgery was performed on all animals during which a unilateral drug cannula was implanted. Possible disruption of different fibers mediating the noradrenergic feeding response was assessed by examining the effectiveness of postsynaptic receptor stimulation through 1-norepinephrine (NE) injections into the paraventricular nucleus (PVN) which elicit feeding in satiated rats; second, PME injections of the presynaptically-acting tricyclic antidepressant protriptyline which elicit feeding through activation of endogenous NE and excitatory lateral paraventricular knife cuts, the medial and lateral hypothalamus which cause hyperphagia and obesity.

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 cannula for all animals in the region of the PVN. The knife cuts were made 4-5 mm lateral to the fornix. They were 4 to 5 mm in height and extended dorsally above the medial-plane of the brain. The electro-lateral plane the cuts were located just medial or just lateral to the fornix.

These findings further demonstrate that the NE elicited feeding response can be dissociated from the hyperphagia syndrome produced by paramedian hypothalamic knife cuts. Furthermore, it appears 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.

The present experiment examines the interrelationship between three hypothalamic manipulations known to increase feeding: first, noradrenergic (NE) injections into the paraventricular nucleus (PVN) which elicit feeding in satiated rats; second, PME injections of the presynaptically-acting tricyclic antidepressant protriptyline which elicit feeding through activation of endogenous NE and excitatory lateral paraventricular knife cuts, the medial and lateral hypothalamus which cause hyperphagia and obesity. Rats received either large paramedian knife cuts rostro-lateral to the VM or sham knife cut surgery. Following recovery a second surgery was performed on all animals during which a unilateral drug cannula was implanted. Possible disruption of different fibers mediating the noradrenergic feeding response was assessed by examining the effectiveness of postsynaptic receptor stimulation through 1-norepinephrine (NE) injections into the paraventricular nucleus (PVN) which elicit feeding in satiated rats; second, PME injections of the presynaptically-acting tricyclic antidepressant protriptyline which elicit feeding through activation of endogenous NE and excitatory lateral paraventricular knife cuts, the medial and lateral hypothalamus which cause hyperphagia and obesity.

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 cannula for all animals in the region of the PVN. The knife cuts were made 4-5 mm lateral to the fornix. They were 4 to 5 mm in height and extended dorsally above the medial-plane of the brain. The electro-lateral plane the cuts were located just medial or just lateral to the fornix.

These findings further demonstrate that the NE elicited feeding response can be dissociated from the hyperphagia syndrome produced by paramedian hypothalamic knife cuts. Furthermore, it appears 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.

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 duplicated many of the effects of bilateral electrolytic lesions. This experiment assessed the effects of LH injections of kainic acid, which appears to damage neuronal afferents while leaving efferent fibers of passage intact. Rats receiving kainic acid injections (0.4 µl/0.2 µl; pH4) were compared with those receiving electrolytic lesions (2 µa, 13-20 min) or sham surgery. The latter three groups showed no significant internal differences and will be collectively referred to as controls.

Postoperatively, both kainic-injected (Kl) and lesioned (EL) rats were aphagic (food intake ≤ 2 g/day) for at least 1 day but, surprisingly, however, were hyperactive to many stimuli. Locomotor activity on the 2nd postoperative day was severely depressed in Kl rats point only to that since kainic brain stimulation at a variety of limbic sites induces water intake. Drinking normally observed after kainic stimulation is blocked by atropine or EL rats. Neither Kl nor EL rats ate above baseline levels following intraperitoneal (IP) administration of 2-deoxy-D-glucose (750 mg/kg), nor did either group show the normal rise in water intake in response to IP injections of hypertonic saline (2 M). Photoplotometric measurement of neocortical and striatal dopamine levels, however, showed content changes among control and Kl groups indicating no damage to monoaminergic fibers of passage. Electrophysiological studies showed many-aminergic changes. Examination of nissl-stained sections revealed kainic-induced cell loss apparently limited to the LH.

A cholinergic coded circuit in the limbic system mediating drinking behavior has been described in Kl rats. By the 4th postoperative day, Kl and control rats were equally mobile and both more active than EL rats. Neither Kl nor EL rats ate above baseline levels following intraperitoneal (IP) administration of 2-deoxy-D-glucose (750 mg/kg), nor did either group show the normal rise in water intake in response to IP injections of hypertonic saline (2 M). Photoplotometric measurement of neocortical and striatal dopamine levels, however, showed content changes among control and Kl groups indicating no damage to monoaminergic fibers of passage. Electrophysiological studies showed many-aminergic changes. Examination of nissl-stained sections revealed kainic-induced cell loss apparently limited to the LH.

The demonstration of alternate patterns of ingestion in suckling rats would be of interest. It would permit direct analyses of the ontogeny of the feeding act and the ingestion act. Accordingly, the capacity of infant rats to ingest in a manner other than suckling was investigated.

Carbachol was injected into the lateral preoptic area of a small piece of papercloth that was saturated with either milk, water, or hypertonic saline (1 M). 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 (1 M, 2% BW, s.c.); (3) 4 hr deprived (pups removed from the mother for four hours prior to testing); and (4) 4 hr deprived + NaCl (1 M, 2% BW, s.c.). Subjects from each treatment condition were allowed to consume either milk, water, or hypertonic saline in a 20 min test.

There were four major findings. First, nondeprived and deprived pups consumed little of any fluid: milk intake: 1-25 µl, water and saline intake: 2-35 µl. Second, acute cellular dehydration increased intake of all fluids. Third, acute cellular dehydration differentially affected water intake, increasing it to 5-78 µl. 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 (1-25 µl) of the saline solution. This selectivity is important since it is a major defining characteristic of adult drinking behavior. Finally, food intake was reduced in response to saline but not milk intake, the latter two showed no age-related differences in the responses of six and ten day old pups.

These findings demonstrate that suckltilke drinking behavior can be elicited by acute 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.

Finally, there were no age-related changes in the amount of water ingested. Thus, while angiotensin and carbachol may both act in the same region, they apparently involve different pathways. Thus, while angiotensin and carbachol may both act in the same region, they apparently involve different pathways. Thus, while angiotensin and carbachol may both act in the same region, they apparently involve different pathways.
EQUIVALENCE OF INTRAVENTRICULAR p-CHLOROPHENYLALANINE AND PHENYLALANINE IN PRODUCING HYPERPHAGIA AND OBESITY IN RATS. Donald V. Gospodac, Brian D. Daniels, 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. 

We report here that intraventricular (i.v.) infusions of p-chlorophenylalanine (pCPA) can induce transient hyperphagia and body weight (BW) gain in rats. Results from our previous experiment 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 lesions fail to elicit overeating or BW gain. Since central infusions of a pCPA congener were not studied despite the quantities (24, 0.7 mg) or 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 intraperitoneal pCPA and phenylalanine (PA) in modifying ad lib food intake. For all experiments, single-housed adult (232-283 g) female Wistar rats were used. Under anesthesia, anesthetized groups of 10 rats received stereotaxically placed i.v. infusions of 2, 3, or 4 mg d,l-pCPA methyl ester HCI or 2, 3, or 4 mg d,l-PA methyl ester HCI in 20 μl saline (pCPA/PA). The pumps delivered fluid to the ventricular space at a rate of 1 μl/hr. During the first day of glycero infusion at a rate of 54 μg/hr into the lateral or third 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 glycero 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 g/day. Infusion of glycero 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 g in body weight during the 7 days of glycero infusion when compared to the body weights of rats receiving intraventricular normal saline at a rate of 1 μl/hr. Infusion at rates of 13 and 7 μg/hr inhibited food intake in a dose related fashion, and led to body weight losses of 100 g and 70 g respectively during the period of infusion. In addition, a significant decrease in pituitary function. The obesity syndrome which follows GTG in male mice has been established. Several investigators have proposed that the hypothalamic monoamine systems may be involved. Damage to monoamine-containing neurons is a well-accepted account for the loss 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 40-60 days of age (25±5 grams). Each male and female were given subcutaneous injections of 0.10 mg/kg. An adult 21 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 day 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 CA were assayed from the base of the skull. The sections were weighed and assayed for norepinephrine (NE) and dopamine (DA). 

In a second experiment, 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 interaction interaction was obtained. Hypothalamic DA was reduced in females but not in males. The correlation between hypothalamic NE and body weight in the GTP groups was not significant.

In the present study, pituitary function was impaired as assessed in the GTG-injected mice of both sexes when compared with saline-injected controls. Furthermore, a significant negative correlation (-1.1, p < 0.001) was obtained between brain NE and PA and weight change. These changes in CA levels may account for the adiposity of the GTP mouse. Recent evidence suggests that the development of obesity in the GTP mouse is pituitary-dependent. 

526 ANOREXIA AND BODY WEIGHT LOSS CAUSED BY INTRAVENTRICULAR GLYCEROL INFUSIONS. John D. Davis and David Wirtshafter, Dept. Psychol., Univ. of Illinois, Chicago, IL 60680.

Food and water intake and body weight were measured in rats before, during and after 7 days of continuous infusion of glycero 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 glycero infusion at a rate of 54 μg/ hr into the lateral or third 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 glycero 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 g/day. Infusion of glycero 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 g in body weight during the 7 days of glycero infusion when compared to the body weights of rats receiving intraventricular normal saline at a rate of 1 μl/hr. Infusion at rates of 13 and 7 μg/hr inhibited food intake in a dose related fashion, and led to body weight losses of 100 g and 70 g respectively during the period of infusion. In addition, a significant decrease in pituitary function. The obesity syndrome which follows GTG in male mice has been established. Several investigators have proposed that the hypothalamic monoamine systems may be involved. Damage to monoamine-containing neurons is a well-accepted account for the loss 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 40-60 days of age (25±5 grams). Each male and female were given subcutaneous injections of 0.10 mg/kg. An adult 21 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 day 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 CA were assayed from the base of the skull. The sections were weighed and assayed for norepinephrine (NE) and dopamine (DA). 

In a second experiment, 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 interaction interaction was obtained. Hypothalamic DA was reduced in females but not in males. The correlation between hypothalamic NE and body weight in the GTP groups was not significant.

In the present study, pituitary function was impaired as assessed in the GTG-injected mice of both sexes when compared with saline-injected controls. Furthermore, a significant negative correlation (-1.1, p < 0.001) was obtained between brain NE and PA and weight change. These changes in CA levels may account for the adiposity of the GTP mouse. Recent evidence suggests that the development of obesity in the GTP mouse is pituitary-dependent.

Supported in part by NRP Grant R58 75-17091.


Salivation was recorded for 6 normal and 6 rats with ventral-medial hypothalamic lesions. Salivation was quantified by placing a cotton-tipped applicator into the rat's mouth and immediately prior to the reintroduction of food into the mouth for one minute. The difference in the pre-injection weight and the post-injection weight of the applicator reflected the amount of salivation. Measures were taken twice daily for 9 days during the post insertion weight of the applicator reflected the amount of salivation. In addition, a significant negative correlation was found between the pre-injection and post-insertion weight of the applicator. The difference in the pre-injection weight and the post-insertion weight of the applicator reflected the amount of salivation. Measures were taken for both normal and VMH animals when compared to normal animals. However, in their study, the animal's responsiveness to natural food or taste stimuli, lemon juice, vanilla, or aniseed solution and water was observed. In the present study, the animal's responsiveness to natural food or taste stimuli, lemon juice, vanilla, or aniseed solution and water was observed. In the present study, the animal's responsiveness to natural food or taste stimuli, lemon juice, vanilla, or aniseed solution and water was observed. In the present study, the animal's responsiveness to natural food or taste stimuli, lemon juice, vanilla, or aniseed solution and water was observed.
THE ORGANIZATION OF FEEDING IN THE DEVELOPING RAT. W. G. Hall.

Plasma calcitonin (CT) concentrations increase following a meal, and the administration of the gastrointestinal hormones glucagon, gastrin, pancreaticoeytin, and cholecystokin. 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 μg/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, s.c.) greatly suppressed feeding for 3 days and drinking for one day (both p<0.01). A retrospective study of humans demonstrated a 23 reduction in 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 rats tested 1 hr before feeding and was 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.


The present study examined the role of dorsal brainstem (DBS) structures in the regulation of feeding behavior by placing bilateral DBS lesions in ten mice, housed individually on a L:12, D:12 schedule. Following at least 15 days of adapta- tion 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. Responses 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 tegmental intact. These rats with lesions in the pontine central gray, but not in other DBS loci, significantly increased their food consump- tion as measured by volume and number of licks. The hyper- phagia was characterized by a significant increase in the number of meals; meal size remained unaltered. Interestingly, the hyperphagic rodents also increased their water intake. This experiment is being replicated using a solid food diet. Dose, 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.

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 (23% 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 shorter in linear growth than controls.

The 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 similarly elevated.

The VMH rats took much longer to achieve body weight levels below the control range for the duration of development, however, they did display finickiness as evidenced by decreased quinine intake, and water intake during food deprivation was decreased through adulthood.

The present results demonstrate that neonatal VMH damage produces relative obesity and finickiness, and the development of obesity awaits further study. Thus, when one views 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 obesity.

Growth hormone and gonadal hormone alteration are likely candidates for the etiology of VMH lesion induced obesity.


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-DG. 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 through adulthood.

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 that to understand its role in the physiological control of drinking, the dynamics of the effectors peptide, AII, must be characterized. We determined plasma AII levels using a radioimmunoassay following experimental treatments that induce thirst.

1) All was infused intravenously (into nephrectomized rats over a period of 10 min). Blood samples were then taken at the end of the infusion. Results are given in the table:

<table>
<thead>
<tr>
<th>Dose of All (mg/kg/min)</th>
<th>1</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AII (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>121.6 ± 7.18</td>
<td>394.6 ± 24.4</td>
<td>843.0 ± 35.2</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>121.6 ± 21.6</td>
<td>242.9 ± 18.6</td>
<td>586.9 ± 35.2</td>
<td></td>
</tr>
</tbody>
</table>

2) Rats (n=28) were water deprived for 12, 24, and 48 hr. Plasma All levels were 136.2 ± 8.6, 117.4 ± 16.1, and 396.6 ± 90.8 pg/ml, respectively (controls with ad lib water 50.2 ± 5.3).

3) Isoproteinol was injected subcutaneously (0.1 and 0.3 mg/kg) in rats (n=15). Plasma All 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 All levels were significantly elevated to 351.0 ± 168.0, 743.0 ± 117.0, and 517.0 ± 100.0 pg/ml, respectively, compared to the levels in sham operated controls (332.4 ± 30.2).

5) Subcutaneous injections of 20X polyethylene glycol (15 mg/kg) in rats (n=21) increased plasma AII levels 15.1-fold, 8.7-fold, and 4.8 fold, and 8 hr to 67.59 ± 11.75, 100.73 ± 17.93, 170.78 ± 18.21, and 349.24 ± 48.40 pg/ml. Saline injections (n=24) of the same volume under the same conditions increased plasma All levels 14.1-fold, 4.13 ± 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 All were elevated to 118.6 ± 11.45 pg/ml, whereas the level in control animals that had no access to dry meal was 6.93 ± 1.7 pg/ml.

The results of these experiments define the range of changes that occur in plasma All levels after several thirst-inducing changes and show that All can act as a dipsogenic stimulus within the physiological range.

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 hypothalamic area (LHA) were made. Within 30 min following KA infusions signs of autonomic and other effects were noted. These included exophthalmia, chorda tympani, vertigo, diaphoresis, alcaptonuria, and behavioral disturbances. The disturbances lasted for several hours post infusion and 50% of the animals died. The Sal infused animals did not show any signs of the above effects. KA infusion decreased food intake by 50% of the animals during the first hr post infusion. Prior to 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 (5 hr test).

Following the infusions, KA treated animals (3µg/0.5µl) decreased water intake on post infusions days 1-2 and 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 infused 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 post infusion food intake at 1 hr testing 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 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 intake, body weights, salt arousal of drinking, post 24 hr deprivation induced eating and drinking in animals treated with 3µg/0.5µl 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.


When milk is delivered directly into the mouth of the infant rat via an introral 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 novel environment (WCC). Infant 2-day old pups allowed to ingest milk presented in discrete pulses consumed 60% of the injected diet (1.82 body weight) compared to 10% when nondeprived rats were tested (Moore and WCC). The behavioral activation consisted of propping, mouting, licking, swallowing, face grooming, rolling over, stretching, twitching, head waging and wall clinging (Moore and WCC) and is not related to 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. Three 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 thepons. Several hours before testing on the next day, the introral cannula was directed into the lateral cerebral ventricle. 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 suprachiasmatic nucleus. Four subjects with cuts ranging from the suprachiasmatic area to the anterior commissure showed decreased food intake and drinking. Six subjects with cuts extending from behind the ventral suprachiasmatic nucleus and behind the ventral suprachiasmatic commissure were not activated (9-18) but had normal intake (17-25). The final group of subjects had activity ratings of 5-10 and normal drinking (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.

VAGOTOMY FAILS TO BLOCK THE SATIATING EFFECT OF FOOD IN THE STOMACH. P. Scott Eraly and James Gibso. Dept. of 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 (Smith and Kraly, 1978). 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 pyloric nooses 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 closed, open 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<0.20, open vs. closed noose) and displayed a postprandial satiety sequence with a mean LR of 11.0 ± 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<0.20, open vs. closed noose) and displayed a postprandial satiety sequence with a mean LR of 11.0 ± 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<0.20, open vs. closed noose) and displayed a postprandial satiety sequence with a mean LR of 11.0 ± 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<0.20, open vs. closed noose) and displayed a postprandial satiety sequence with a mean LR of 11.0 ± 0.4 min.

These results confirm the findings of Eraly and Smith (1978) and show that a satiety signal of gastric origin, when combined with pregastric stimulation by food, produces normal MS and LR. These results also show that gastric vagotomized rats with pyloric nooses ate liquid food after 3-hr food deprivation. When the noose was open, vagotomized rats ate a mean MS of 4.2 ± 0.6 ml and displayed a postprandial satiety sequence with a mean LR of 10.9 ± 1.6 min. When the noose was closed, vagotomized rats ate a mean MS of 4.2 ± 0.6 ml and displayed a postprandial satiety sequence with a mean LR of 10.9 ± 1.6 min. When the noose was closed, vagotomized rats ate a mean MS of 4.2 ± 0.6 ml and displayed a postprandial satiety sequence with a mean LR of 10.9 ± 1.6 min. When the noose was closed, vagotomized rats ate a mean MS of 4.2 ± 0.6 ml and displayed a postprandial satiety sequence with a mean LR of 10.9 ± 1.6 min.

Supported by funds from the Graduate School of Arts and Sciences (NJK), AM-18744 (SCW) and the Kroc Foundation (RHW).

Bilateral electrolytic lesions of the lateral hypothalamic (LH) area of rats produced transient hypokinesia, poor postural support, ataxia, abnormal spiking and slow wave activity in the hippocampus, and abnormal slow wave activity in the neocortex lasting 1 to 5 days was observed in 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 10 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 free running locomotion in the hippocampus or theta rhythm, or the appearance of atropine-sensitive hippocampal theta activity seen after electrolytic LH lesions. KA injections caused a transient increase in the slow wave activity which electrolytic lesions did not. The results suggest that destruction of cells intrinsic to the LH contributed to these behavioral deficits and some transient abnormal EEG activity. However, chronic changes in water intake regulation and EEG activity observed only after KA lesions probably result from the exposure of cells to the result of damage to axons passing through the LH.


The pentobarbital intracerebroventricular infusion of saralasin on feeding behavior in non-primate species. The monkeys were first acclimated to cable restraint and baseline feeding behaviors were studied under identical conditions. The effects of intermittent LC stimulation, previously described, served to occur during stimulation. Stimulation did, however, persist for 5-10 min of water access was 1.96±0.31 and 1.19±0.36, and at 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given 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 for the first 75 min of the experiment. The rats 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 3 h 3.46±0.55 and 2.18±0.50, respectively. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.630±0.36 ml/100 gm (n=9) in the 90 min following hypotonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in rats given saline controls, intakes that were not significantly different.
EFFECT OF ANTEROVENTRAL THIRD VENTRICLE (AV3V) LESIONS ON BODY WEIGHT IN GENETICALLY OBESE MICE. Joan F. Lorden. Dept. Psychol. and Neurosci. Prog., Univ. Alabama in Birmingham, Birmingham, AL 35294.

Similarities between genetically transmitted obesity and hypothalamic lesion-induced obesity have suggested the possibility that the hypothalamic lesion may be the lesion responsible for obesity in obob and ddmb mice. In addition to obesity and concomitant metabolic changes, both obob and ddmb mice have numerous neurological anomalies suggesting either anterior pituitary malfunction or a failure in hypothalamic control of the pituitary. No naturally-occurring hypothalamic lesions have been reported in either obesity. The elevated levels of hypothalamic and telencephalic norepinephrine (NE) have been reported in both mutants when compared with lean littermate controls. Central norepinephrine lesions 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. It is possible to assess the altered CA levels in the obesity syndromes of obob and ddmb mice, intraventricular infusions of 6-hydroxydopamine (6OHDA) were used to reduce CA levels in the brains of both mutants.

Female ddmb mice (C57Bl/6J or male and female obob mice (C3H/BlJ) 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) 6OHDA.HBr, dissolved in a .9% saline-0.2% ascorbic acid vehicle. The 6OHDA was delivered in a .2 µl volume through a 30 gauge cannula, stereotactically aimed at the third ventricle. Lean and obese control animals received 0 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 either lean or obese animals. The lesions significantly reduced both body weight (-15%) and blood glucose (-24%) in ddmb mice when compared with vehicle-injected obese controls. Neither lean 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.

Assays of hypothalamic and telencephalic NE and DA showed that the 6OHDA infusions produced large decreases in the levels of both amines. The infusions in the obob mouse did not decrease in lean mice or obob mice was not due to a failure to produce a lesion. The results indicate that despite the similarity between the two syndromes, central monoamine systems play different roles in the development of obesity in these mutants.

### Table: Food Consumed (Mean ± S.E.M of Saline Control)

| Group               | Complete Vagotomy | Control Vagotomy | Complete Section | Control Section | Controls
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>CCK</td>
<td>Oct</td>
<td>CCK</td>
<td>CCK</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>204/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>124±9.7</td>
<td>25±15.0</td>
<td>53±12.0</td>
<td>54±10.8</td>
<td>144±7.1</td>
</tr>
<tr>
<td>400/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>102±6</td>
<td>26±14.7</td>
<td>54±12.7</td>
<td>59±9.0</td>
<td>138±10.1</td>
</tr>
<tr>
<td>800/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>94±6</td>
<td>7±10.7</td>
<td>70±14.5</td>
<td>59±15.1</td>
<td>81±6.3</td>
</tr>
<tr>
<td>1600/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>84±8</td>
<td>31±3.0</td>
<td>-</td>
<td>-</td>
<td>90±4.2</td>
</tr>
<tr>
<td>3200/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>114±26.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6400/µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>121±17.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: * = % increase, ** = % decrease

Supported by NSF SHT7-12394 and NIHCS 03469.

Angiotensin II (All) has been shown to be a potent dipson in all adult species tested. This study examined the ontogeny of the water drinking response to All in the first two hundred days of life. One hundred ng of All (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 5 day old litters. Animals were tested for weight with the All treated rats received 1 μl of the vehicle alone. Responsiveness to All was evaluated by a modification of Wirth and Epstein's technique (1976 Amer. J. Physiol. 230: 180-198), which uses weight gain during the test period as a measure of a water ingested by compulsory 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 All 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 All were seen at 4 days of age. At 5 days the weight gain due to water drinking after All was double that at 4 days (All = 17mg ± 65, n = 13 vs. control = 17mg ± 9, n = 12). Responsiveness to All 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 milk, was offered to 5 day old All treated rats, they drank significantly more than controls (All = 318mg ± 23, n = 8 vs. control = 15mg ± 4, n = 12). When milk 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 All in control of suckling behavior rather than at a later phase, dipsony, because 24 hour old rats treated with All 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 All 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 NINDS 03469 and MH 28782.)


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 (Edwards and Hough, J. Endocr. 65: 99, 1975). ACTH and β-endorphin are released concomitantly in the rat (Gullemin 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 Gudeliti, 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 a.c. of naloxone hydrochloride in C57BL/6J ob/ob, Beatriz Heister and I. L. Margules, Purdue Univ. West Lafayette, IN 47907. It has been reported recently that chlorisondamine (CORM), librium, clonidine, and cyproheptadine increase food intake in non-food deprived rats (NFD) (Yim et al., Fed. Proc. 47(3): 860, 1977). There are afferents to the SON from the OVLT and to the NMD as well. These connections may subserve the physiological function of All in the central control of water balance.


We have recently reported that chloriscondamine (CORM), 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 μg/5 ul i.v.c.), clonidine (5.0 ± 5.0 mg/Kg, i.p.), and theophylline (10 mg/Kg, i.p.) increased daytime 3 hr food intake by 76, 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 250 percent, clonidine (5.0 mg/Kg, i.p.), and theophylline (5 mg/Kg, i.p.) increase food intake by 44 percent (control = 16.3 ± 2.5). In NFD rats tested at night, the same doses of NE, clonidine, CORM, theophylline, and cyproheptadine increased daytime food intake by 27-47 percent (controls = 7.3 ± 0.1 g/Kg). These results suggest that the increased daytime feeding induced by the brain agent (in a receptor specific manner) is not a result of the ability to produce a phase shift in the circadian feeding rhythm of the rat. Supported by grants from NIH (NS 10277, 5-RO1CA14782), 09 and EPA (R-801965).
THE ROLE OF SOMATOSTATIN (SRIF) IN THE CONTROL OF FEEDING.


In Part I (in press), we showed that somatostatin (SRIF) is secreted into the hepatic-portal (HP) circulation and that HP infusions of SRIF can alter food intake. In the present study, we investigated the effects of SRIF on food intake in rats subjected to various metabolic conditions.

We infused rats with HP infusions of SRIF during the period of nocturnal overeating or following cyclical food deprivation. 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 food intake 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, HP infusions of 300 ug of SRIF (300ug/hr) produced a significant (p<0.05) and long lasting suppression of FI without producing other behavioral changes. HP 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 exogenous angiotensin II and/or hypertonic saline (Johnson & Winnick, Am. J. Physiol. 233:R44, 1977). Supported by NIH Grants 15455, AM17240 and MH00149.

Supported by MRC Canada.


Feeding in response to 2-deoxy-D-glucose (2DG) or insulin is presumed to be mediated by circulating CCK. 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 two indwelling intragastric catheters and a jugular venous catheter (Johnson & Winnick, Am. J. Physiol. 233:R44, 1977). 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 two indwelling intragastric catheters and a jugular venous catheter (Johnson & Winnick, Am. J. Physiol. 233:R44, 1977).

Supported by MRC Canada.


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. 233:R44, 1977). Further, these animals do not become hypertensive or increase water intake following the CCK release (Buggy, Plass, Johnson & Brody, Circ. Res. 40:110, 1977).

Animals which have recovered from the acute effects of AV3V lesions drink normally in response to 250 g of water deprivation but fail to increase water intake in the 4th hour following caval ligation.

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 peptide nature of CCK we conclude that under these conditions 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 vagus nerve. 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/kg), 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/kg in unilaterally vagotomized rats and 10 U/kg in intact rats. Since bilateral vagotomy (hepatic branch preserved) abolished the satiety effect of doses of CCK-8 <40 U/kg, we conclude that under these conditions low doses of CCK-8 act to elicit satiety at a extrahepatic abdominal site innervated by the vagus nerve.

Supported by NIH Grants MH13545, AM17240 and MH00149.

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 NIMH Grants GM21728 and MH 11218)


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 food intake following intragastric administration 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 control of food intake are altered with changes in photoperiod. 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).

INTRAHYPOTHALAMIC INJECTIONS OF KAINIC ACID PRODUCE DEFICITS IN FEEDING AND DRINKING DURING ACUTE HOMEOSTATIC IMBALANCES. Edward J. Stricker, Alissa F. Svedroff, and Michael J. Zigmond. Departments of Psychology and Biological Sciences, University of Pittsburgh, Pittsburgh, Pa 15260.

Glycerol injections into 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 ventromedial hypothalamus. However, it is now known that LH lesions interrupt dopamine-containing neurons ascending from the mesencephalon to the forebrain, and that specific destruction of these 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 near the injection site while sparing fibers in the vicinity of the injections. Glycerol injections into the far lateral hypothalamic area of rats 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 (1.14 M NaCl, 3 ml, i.p.) was found to be abolished in 4 animals and impaired in 1 other; 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.)

A controversy exists with regard to the mechanism by which an increase in the extracellular osmolarity of extracellular fluids stimulates thirst. The osmometric hypothesis of thirst is based on Verney's (Proc. Roy. Soc. Lond. Ser. B, 135, 25-106, 1947) suggestion that there is an increased withdrawal of water from sensitive receptor cells within the hypothalamus and lead to vasopressin release. Alternatively, Anderson and coworkers (J. Physiol. 59, 404-425, 1961) have postulated that existence of receptors sensitive to the concentration of sodium in cerebrospinal fluid (CSF). As a test of the Anderson mechanism we infused 3 conscious dogs with hypertonic, osmolality is mediated by an osmoreceptor mechanism. Furthermore, sucrose infused centrally initiated drinking. The only solutes which stimulated drinking were those which penetrated cell membranes, stimulated thirst and increased CSF sodium conc. by 1.650.3 and 1.250.3 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 osmoreceptor mechanism, we decided to increase the CSF osmolarity 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, glucose, urea or isotonic NaCl iv and measured the change in CSF sodium conc. at the thirst threshold or after a recent injection, after 45 min. Hypertonic NaCl, 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. 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.

562 THE EFFECTS OF COPULATION ON FEEDING AND RELATED PROCESSES IN THE MALE. Freya A. Weizenhoff, 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 (SA) mated every other week (1 mating/test). The other 6 males comprised the second group (SR), which did not mate during the following 6 weeks. At the end of the 6 week period, body weight and fat 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 overconditioned female.ROLS used by SA and SR groups were tested individually 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 effects of pairing a male with an estrous female that was different from pairing with an overconditioned rat. The SR males tended to show increased food intake following sexual behavior, but only if they were paired with the female. These results suggest that social interactions can alter regulatory processes in the male. Other have shown that sexual behavior induces increase in testosteron. It is known to alter food intake in males (J. Comp. Physiol. Psych. 90: 18, 1976), and testosterone-sensitive tissues (Physiol. Behav. 9: 40, 1972). Testosterone-sensitive females. The present studies extend the previous experiments concerned with the effect of the female on reproductive functions in the male because present studies do not test testosterone-sensitive processes are also altered by exposure to female conspecifics.

563 RAPID 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 92039

In the search for understanding the role of the serotoninergic neurotransmitter system in appetite regulation, earlier studies by several investigators have suggested a reciprocal relationship between food intake and serotonin metabolism. However, misinterpretation of such reports is made more complex by our recent finding that graded doses of tryptophan that more than doubled brain serotonin synthesis, doubled food intake. Other studies have not reported the same phenomenon. 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 conveyed by descending and serotonergic afferent, rather than by serotonin biosynthesis, may be the pertinent serotonergic factor in the modulation of appetite. Using a number of agents active on the serotoninergic 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 appetitive behavior is partially reversed by tryptophan, which increases serotonin synthesis, when both drugs are administered simultaneously. Changes in patterns of serotonin's modulatory action on appetite: 1) the serotonin cells may act from outside the primary appetite informational circuit to regulate transmission along this pathway by altering the synthesis of serotonin. 2) they may release a neurotransmitter contained within the primary pathway for the neuronal representation of appetitive 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.


Previous studies have demonstrated that suckling behavior of infant rats is not an unconditional reflex but that many of its components develop and change prior to weaning. Before weaning, motivation toward suckling is set by several factors including: 1) the female's presence, 2) the suckling stimulus itself, and 3) maternal deprivation. Methysergide (MST) is a serotonin antagonist that inhibits the effects of the female's presence and the suckling stimulus itself. In addition, MST blocks the effects of maternal deprivation. These observations have led to the hypothesis that social interactions with others can alter the appetite of young rats.

In order to test the effects of methysergide on the components of suckling involved in milk intake, we utilized an intracerebral 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. We have recently found that pharmacological manipulations of the serotonin system can alter the initiation component of suckling. For example, when the mother is removed from the cage, the maternal deprivation blocker methysergide reinstates suckling in non-deprived pups older than 15 days. If suckling were a unitary behavioral system, then this pharmacological manipulation should similarly reinstate other components of suckling commonl 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 intracerebral 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. We have recently found that pharmacological manipulations of the serotonin system can alter the initiation component of suckling. For example, when the mother is removed from the cage, the maternal deprivation blocker methysergide reinstates suckling in non-deprived 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.
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
INSIGHTS INTO THE MORPHOLOGICAL BASIS OF PRESYNAPTIC FACILITATION

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 proposed, produces an instability that maintains bursting activity as negative losses to the cell can actually "see" the negative resistance during normal activity, i.e. that a small depolarization leads to an increased inward current flow, 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 burst progresses, 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.

INSIGHTS INTO THE MORPHOLOGICAL BASIS OF PRESYNAPTIC FACILITATION IN THE GILL-WITHDRAWAL REFLEX OF APLYSIA: ANALYSIS OF THE FINE STRUCTURE OF A MODULATORY SYNAPSE.

Hawkins et al. (1976, 1977) have identified a group of neurons (L28, L29) in the abdominal ganglion of Aplysia which produce presynaptic facilitation in responses between sensory and motor neurons of the gill-withdrawal reflex. It has been postulated that these facilitatory neurons may be serotoninergic. The structure of these facilitatory neurons on sensory neurons is of interest 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 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 synapses. 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. (1976, 1978). Both the serotoninergic vesicles of the GCN and the vesicles of the facilitators resembles vesicles found by Bailey et al. (1976, 1978) in terminals which synapses on gap-junctions in Aplysia nerve cells. 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 burst progresses, 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.

THE SALIVARY BURSTER OF LIMAX MAXIMUS: A PRESUMPTIVE SENSORY-MOTOR NEURON.

Gel filtration chromatography shows the substance to have a molecular weight in the range 5000-8000. Gel isoelectric focusing shows AGF to be a water-soluble substance which is sensitive to exposure to 1% sodium dodecyl sulfate, M urea, and 0.5% Triton X-100, and to be precipitated from neutral aqueous solution by addition of (NH₄)₂SO₄ to 40% final concentration. Gel filtration chromatography shows the substance to be a protein.

Insects are thought to lack a mechanism for directly detecting spatial orientation. Instead, tight, 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. Physiological experiments in our lab indicate that two rows of pendulous sensilla found on each cercus of the burrowing cockroach, Arenivaga, provide equilibrium information.

The plum 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 byafferents 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 #NSF77-22283 and N A V A N A X I N E R M I S. M. V. L. Bennett, D. C. Spray, M. E. Spira* 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 and motorneurons controlling pharyngeal expansion. During high rates of discharge evoked tactilly or by electrical stimulation impulses may fail to invade the soma. Failures may occur at different dates 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 induced by CI- injection. Thus, chemical mediation is indicated. The buccal region innervated by a sensory neuron may have an excitatory or an inhibitory region neighboring it or surrounding it. 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.

DSC is a McKnight Scholar.

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 (ereon: T.H. Bullock). Biology Dept. B-022, UCSD, La Jolla, C A 92039.

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. Gasielle and D.K. Hartline (in preparation) have shown that ipsp's from the AB neuron have a rapid 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 innervated by a sensory neuron may have an excitatory or an inhibitory region neighboring it or surrounding it. 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.

Supported by National Science Foundation grant #NSF77-22283 and National Aeronautics and Space Administration grant #N5C-7435.

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 integration and firing behavior. 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 (Shafrin et al., this volume). As a result there is a several second silent period of 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 ion 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, capacitative 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 lat 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 role of the delay in 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 elements to the features of the behavior which those cells mediate. Supported by NIH grants NS1311 and NS09200.

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 in diameter) were 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 responded in density.

Yet another possible difficulty is pharmacological effects of the dyes. Suction electrode recordings from the connective and antennal nerve were used to monitor neurons that respond 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 response 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 antennal nerve were used to monitor neurons that respond 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 response 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 antennal nerve were used to monitor neurons that respond 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 response 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 antennal nerve were used to monitor neurons that respond 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 response 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.
FUNCTION OF MOLLUSCAN SALIVARY NEURONS DURING FEEDING. Jonathan Copeland* and Alan Gelprin (SPON: M. A. Lamper). Department of Biology, Princeton University, Princeton, New Jersey 08540.

The salivary burster (SB) is an autonomic motoneuron which initiates contraction of the salivary duct of the giant garden slug, Limax maximus (Prior and Gelprin, 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 to 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 gland cell (MGC). The BSNs and MGCs 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, 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 extracellular EPSPs from NP, SN3, another slow bursting neuron, also have an effect on the salivary duct. SN3 is not affected by MGC stimulation. 

These results suggest that 5-HT is involved in the transmission of phase shifting information within the eye. Release of transmitter(s) that does not contain serotonin causes phase shifts in an excitation-free in vitro system. The salivary gland cells respond to serotonin as well.


In studying the reception of temporal information by the circadian pacemaker (CP) of the aphid, we have 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 (10-5M) produced a 1-hr. phase advance in the CAPs when the treatment was administered late in the subjective day. At some other phases, 5-HT produced delayed phase shifts. Phase shifts and inhibition of the CAPs were produced by concentrations of 5-HT as low as 10-7M. Other putative transmitter substances, dopamine and alpha-aminobutyric acid, do not cause phase shifts in the rhythm at phases where 5-HT is effective. However, butyramine (10-5M), 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 electrically coupled to it, since solutions capable of inhibiting secretion (HgCl2, KCl, NaCl) did not block the 5-HT phase shifts.

If 5-HT performs a neurotransmitter role in the eye, 5-HT should be present in the eye with some portion of it in a releasable state. The eye contains a substantial amount of 5-HT and 5-HT-3 receptors, which are similar to the 5-HT receptors found in the skin. The 5-HT receptor in the skin is a photosensitive receptor, which is sensitive to the light emitted by the eye. The 5-HT receptor in the eye is a photoreceptor, which is sensitive to the light emitted by the eye. The 5-HT receptor in the eye is a photoreceptor, which is sensitive to the light emitted by the eye. The 5-HT receptor in the eye is a photoreceptor, which is sensitive to the light emitted by the eye.

Supported by NSF Grant BNS 75-04637.
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 group associated with the ventral group. Control of the excitability of the VNC and ventral groups has been investigated intracellularly in deafferented preparations and during tetanized walking. Dorsal GI's are excited during walking while ventral ones are inhibited. (Herricks, 1971, J. Neurophysiol. 34: 317–329.)

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 the same GI in a bilaterally transected VNC cut into two hemiganglia. The results of these experiments suggest that the major source of walking-activated excitation is from sensory neurons in the thorax. 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 co-motoric 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 isolating experiments were performed. Deafferentation of thoracic and abdominal ganglia (many of the cerebral nerves were intact) guaranteed sensory input associated with walking was eliminated. Yet during leg motoneuron activity sound responsiveness of thoracic 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 resting animals with these pathways 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 thoracic motoneurons GI's receive inhibitory input from both central and peripheral sources.

Supported by NSF Grant BNS 721721

INVERTEBRATE NEUROBIOLOGY

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 group associated with the ventral group. Control of the excitability of the VNC and ventral groups has been investigated intracellularly in deafferented preparations and during tetanized walking. Dorsal GI's are excited during walking while ventral ones are inhibited. (Herricks, 1971, J. Neurophysiol. 34: 317–329.)

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 the same GI in a bilaterally transected VNC cut into two hemiganglia. The results of these experiments suggest that the major source of walking-activated excitation is from sensory neurons in the thorax. 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 co-motoric 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 isolating experiments were performed. Deafferentation of thoracic and abdominal ganglia (many of the cerebral nerves were intact) guaranteed sensory input associated with walking was eliminated. Yet during leg motoneuron activity sound responsiveness of thoracic 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 resting animals with these pathways 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 thoracic motoneurons GI's receive inhibitory input from both central and peripheral sources.

Supported by NSF Grant BNS 721721

INVERTEBRATE NEUROBIOLOGY
BAG CELL HORMONE ACTS DIRECTLY ON OVOTESTIS OF APLYSIA CALIFORNICA IN VITRO: BIOASSAY FOR RELATING RELEASE TO ELECTROPHYSIOLOGY. D. J. Dockery, G. E. Searle, and S. S. Tobey. Erindale College and Department of Zoology, University of Toronto.

The bag cells of Aplysia fire a characteristic train of action potentials to release the peptide hormone bag cell hormone (BCH) when triggered to fire. It has been hypothesized that the neurohormones act directly on the ovotestis to initiate egg release. We have found that extracts of the parenchymal tissue (PVG) with 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 the ovotestis do not increase egg release above control levels in artificial sea water (ASW), thus indicating that nacromolecules associated with 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.

California 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 (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 electrolytically-evoked afterdischarge 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.

ROLE OF INTERNEURON II IN LONG-TERM SENSITIZATION OF SIPHON WITHDRAWAL IN APLYSIA CALIFORNICA. Lewis Eberly and Harold Pinsker. Dept. Physiol. and Biophysics, Marine Biomedical Inst., UTM3, Galveston, TX 77550.

Aplysia show spontaneous, stereotyped contractions of the siphon which may be elicited by a trigger as well as by water jet. This behavior is mediated by a network of interneurons in the abdominal ganglion called Interneuron II (INT II) (Byrne and Kandel, 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 Kandel, 1976). Plastic changes in latency of triggered INT II activity can contribute to short-term habituation and sensitization of the siphon withdrawal reflex (Kan, 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 5-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 60E to 85E) in the probability of a fifth withdrawal 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 stimulation, whereas controls show either no change or an increase. The greater frequency of occurrence and reduced latencies of triggered INT II siphon in sensitized animals is presumed to play a role in the behavioral changes associated with sensitization.

Triggered INT II responses play a significant role in siphon withdrawal for several reasons. (1) Spontaneous INT II responses lead to a nearly complete 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.
POSITION LEARNING IN BEHAVIORALLY APPROPRIATE SITUATIONS.

W. Forman* and C. Hougl., Dept. Biol., Univ. of Oregon, Eugene, OR 97403 USA. Supported by NSF Grant BNS-75-00463; PHS 5-T-32 GM 07257.

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 intra-cellularly 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 a device that brought food to the mouth, regular feeding was learned, the segment threshold at intervals to turn the heat lamp on and off to maintain a preferred temperature. Increments and decrements in temperature. All feeding time increased to 2-7 min at an average interval of 45 min. All animals 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.


Parasitic contractions and other muscular movements which appear to function in evulsion 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^{-6} M) and acetylcholine (10^{-5} M) inhibit spontaneous contractions and decrease the resting tension of the muscle. 5-Hydroxytryptamine (10^{-4} M) increases the rate of spontaneous contractions.

Upon penetration of the ventral segment of an isolated male schistosome with a microelectrode a potential difference (-31.52±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 osmotic concentration has the greatest influence on both the tegumental membrane potential and the resting tension of the muscle. 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 rhythmic activity 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 an isolated whole larva 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.)

TEMPERATURE EFFECTS ON THE ELECTRORETINOGRAPHIC CIRCADIAN RHYTHM IN CRAYFISH. Beatriz Paredes-Peñas and Javier Ramos C.* Depto. de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México 20, D.F. MÉXICO

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 underlying 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 C 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.

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 the transmitter, and packaging 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 3H-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. 3H-Dopamine and 3H-histamine both are transported in amounts and at velocities similar to 3H-serotonin. With both substances, the radioactivity appearing in the axons has the same distribution pattern as the pattern of the injected transmitter. 3H-Dopamine and 3H-histamine are transported when injected in small amounts, but there is virtually no transport when much larger amounts are injected. When unlabeled D-3H-L octopamine is injected together with 3H-serotonin, the subsequent transport of the latter is partially depressed. 3H-choline, when injected together with 3H-serotonin, is converted into 3H-phosphorylcholine and 3H-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.

The effects of dopamine on dopamine-sensitive neurones in the brain of Helisoma trivolvis. Bonnie Granzow and Stanley B. Kater. Apr. 26. Zool. 191:193, 1975). The brain-specific substance is released, during high-% depolarization, by a [Ca++] dependent mechanism (Goudsmit, Brain Research, 1977). The dopamine-sensitive neurones in the brain of Helisoma trivolvis show a 2- to 10-fold increase in the specific activity of galactogen when compared with duplicate explants from the same animal, and in the absence of dopamine, the cerebral glands are inactive. Therefore, it is unlikely that the brain-specific substance released in the cerebral glands itself is an activator of galactogen synthesis. The presence of a single brain cell culture in the organ culture is sufficient for galactogen synthesis, and it is proposed that the brain-specific substance released in the cerebral gland is a galactogen-stimulating substance possessing properties characteristic of known peptide neurohormones. Supported by NIH grant GM-23240.

EVIDENCE FOR NEUROHORMONAL ACTIVATION OF GALACTOGEN SYNTHESIS IN THE ALBUMEN GLAND OF THE SNAIL, Helix pomatia. Esther M. Goudsmit, Ralph J. Greenspan, James A. Finn, Jr. and Jeffrey C. Hall. Dept. of Biology, Brandeis Univ., Waltham, MA 02154.

Our recent studies have shown that the brain-specific substance retains activity after exposure to 100ºC for six minutes, but not after treatment with protease. Galactogen-14C is synthesized at a high rate in the albumen gland explants at 100ºC for 6 minutes, but not after treatment with protease. Therefore, it is unlikely that the brain-specific substance is an activator of galactogen synthesis. Concentrations of 5-10-7 M have no stimulatory effect. The amount of galactogen released by a single cerebral cell is 2-4 fold greater than the amount of galactogen released by a single cerebral cell. Therefore, it is unlikely that the brain-specific substance released in the cerebral gland is an activator of galactogen synthesis. The amount of galactogen released by a single cerebral cell is 2-4 fold greater than the amount of galactogen released by a single cerebral cell. Therefore, it is unlikely that the brain-specific substance released in the cerebral gland is an activator of galactogen synthesis. The amount of galactogen released by a single cerebral cell is 2-4 fold greater than the amount of galactogen released by a single cerebral cell.
A search for unidentified neuron(s) may become exceedingly difficult even in large nervous systems, since many pairs of cells have to be impaled, or if the neurons are small. A such a search might be easier if it was possible to stimulate neurons with focal laser light; this scanning of the large micropipette 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 cerebral ganglia of the leech, Hermodice carunculata and the superasaphis ganglia of the barnacle, Balanus mulluscius. A neuron was impaled with microelectrode to monitor its electrical activity. The ganglion was then perfused with normal Ringer containing 100-1000 mg/ml of a photoreactive compound (indolelabeled tiothiatricaric 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 μm which was positioned on the impaled neuron with the aid of 40X/.7NA Zeiss water-immersion objective to provide vertical 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 M 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 resting potential of the ganglion 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 if did, it did 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 the laser is localized in the illuminated area. It seems that the in-plane resolution for stimulation is 4–6 μm, and the depth of field is 5–10 μm. Thus small neurons probably can be identified in the leech, when shorter light pulses were used.

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 2 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, if certain 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 orientation of the photoreactive compound. Supported by a fellowship from Muscular Dystrophy Association.

We have purified a vasopressin-like factor from Helix 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 2 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, if certain 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 orientation of the photoreactive compound. Supported by a fellowship from Muscular Dystrophy Association.

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 2 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, if certain 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 orientation of the photoreactive compound. Supported by a fellowship from Muscular Dystrophy Association.

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 2 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, if certain 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 orientation of the photoreactive compound. Supported by a fellowship from Muscular Dystrophy Association.
SOCIETY FOR NEUROSCIENCE


Sensitization of the gill-withdrawal reflex in aplysia is due to a 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. The inhibition of one of L29 cells 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 markers into a facilitating neuron now allows a morphological analysis of how these widespread modulating effects are produced.


Experiments designed to study inhibition in the closer muscle of the claw and ventral leg of Hymenocera nobilis revealed that the inhibitory innervation differs from that previously described for the superfamilly Nephropidae. 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 the 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-epidermal region, antidromically-evoked IPSP's were elicited in the bender and extensor muscle fibers. 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 hypopolarizing IPSP's were found only in specific areas of each of the three closing or inhibitory axons. Antagonistic muscles are inhibited during contraction of agonists.

Supported by grants from NSF and NIH.

603 IONTOPHORETIC MAPPING OF ACETYLCHOLINE RECEPTORS ON THE SONA, AXON AND IN TWO DISTINCT NEUROEPHES OF A GIANT MOLLUSCAN NEURON. Philip E. Hockerberg 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, seemingly conflicting information on the distribution 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 specific 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 Murrill, J. Exp.Biol.15: 727-736, 1970) which can be readily investigated. The ability to directly visualize 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 -7 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 nerve-transmitter does not eliminate the transmitter from consideration. Additionally, it appears that molluscan neurons may not be as non-localized as previously thought. (Supported by NIH grant #NSF SER 76-18255.)

604 AXONAL ORGANIZATION OF THE DORSAL MESOTHORACIC NERVE INNERVATING THE DORSAL LONGITUDINAL FLIGHT MUSCLE OF DROSOPHILA MELANOGASTER. Kamo Iikeda and Takashi Tsurushina. City of Hope Natl. Medical Center, Duarte, CA 91010.

The arrangement of motor axons controlling the longitudinal flight muscle (DIM) was studied with light microscopic (K.L.) and electronmicroscopic (T.T.) methods. The six bilateral pairs of DIM fibers are innervated by five axonal bilaterial pairs from the anterior dorsal and posterior dorsal nerves, as was previously reported. These DL M fibers are referred to as 45-a through f from dorsal to ventral (Miller, 1950) and correspond to DIM 6 through DIM 1 in our previous report. These DIM fibers are innervated by five bilateral pairs of antagonistic muscle fibers, 4) 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 hypopolarizing IPSP's were found only in specific areas of each of the three closing or inhibitory axons. Antagonistic muscles are inhibited during contraction of agonists.

Supported by grants from NSF and NIH.
NEUROTRANSMITTER MODULATION AND \textit{cAMP} CORRELATES OF
activation of adenylate cyclase is linked to bag cell activity.

After a brief period of refractoriness, the excitability may revert to its
normal level. This period of refractoriness or afterdischarge is also
associated with a specific increase in total
refractoriness. We have shown that both serotonin and dopamine increase
refractoriness (type II) controls the duration of afterdischarge in the
neuroendocrine bag cells of Aplysia. We have distinguished two apparently
independent types of refractoriness. The
first (type I) is characterized by a rapid firing rate within 1 min of the
onset of each afterdischarge. Type II refractoriness may be
prevented by increases in temperature or by partial substitution
for extracellular chloride ions. The second form of
refractoriness (type II) may be
expressed in the isolated asomatic neurites of the bag cells. We
have shown that this form of refractoriness is
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.


e

INCREASE IN PRESYNAPTIC CALCIUM CURRENT ASSOCIATED WITH PRE-
SYNAPTIC FACILITATION MEDIATING BEHAVIORAL SENSITIZATION OF THE
GILL WITHDRAWAL REFLEx IN APLYsIA. M. Klein,* and E.R. Kandel.
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 (sen-
sitizing) stimulus. This type of sensitization results from a
facilitation that is sometimes called presynaptic facilitation.

These results are consistent with the idea that presynaptic
facilitation can be simulated with extracellular injection of
serotonin (5-HT) or with intracellular injection of
cyclic AMP (cAMP). The facilitation that was observed in
a 5-HT-sensitive adenylate cyclase in the presynaptic mem-
XL, 855). In the nerve of Ascaris, the presynaptic
facilitation results from a CAMP dependent increase in the Ca++
ion triggered by the action potentials of the

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 0.6 M tetraethylammonium (TEA). In the
sensory nerve 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,
specifically at the circumesophageal connective (CEC). In TEA
in 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,
specifically at the circumesophageal connective (CEC). In TEA
in the sensory neuron action potential (normally 2-4 msec) shows a
slowly repolarizing plateau of about 50 milliseconds duration.

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 nonsniping somata and sniping axons. Pitman et al. (Science, 1972, 178, 507) recently demonstrated that nonsniping somata of cockroach motoneurons will support spikes following axotomy or implantation of colchicine; a finding that has been replicated in the locust (Goodman & Hattler, 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 to be examined 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 nonsniping somas, 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 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 abdomen serves as a 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 in this case 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 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 the soma.

Burst mode of the L cell.

10mV
2sec


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 histochemical properties among fibers of a muscle which contains all long-sarcemere fibers. This variability is correlated with the pattern of innervation by the fast and slow motor axons.

The closer muscle of the 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 high 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 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 high 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.

Supported by grants from NSF, Muscular Dystrophy Assoc. and by a NIH-NINDS Research Career Development Award (to P.L.).

During late winter and spring months the neurons in genital ganglia from A. californica were typically silent and exhibited accommodation to maintained intracellular depolarization. Stimulation of the genital neurons and 3) involved with alterations in reproductive activity (Strumwasser et al., Comp. Biochem. Physiol. 25:197, 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. brasiliensis 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 electrophysiological states is 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 genital ganglia was observed after bag cell excitation in vivo. 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 hemaphroditic duct caudal to the spermathecal duct). Homogenates of the atrial gland were able to release eggs from isolated reproductive tissues, 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 transitions in some cells were characteristic of 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 (JTL), NS05830 (JTW), NS07613 (JEB), and NSF grant PCM76-18936.

614 BIOCHEMICAL IDENTIFICATION OF SEROTONIN IN VITAL-STAINED NEURONS FROM LEECH GANGLIA. Charles M. Lent, Kent P. Kuyper and Joyce Ong. SUNY Stony Brook 11794 and City of Hope, Duarte, CA 91010.

Seven widely-scattered neurons in leech segmental ganglia fluoresce yellow following Falck-Hillarp treatment. The fluorescence in the two colossals (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. Natural 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 Macrobella decora by an enzymatic technique (Sawada et al., J. Phys. Exp. Therap. 186:506, 1973) which is sensitive to 50 femtomoles of serotonin (5X10^-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 pmoles. Staining of the Retzius cells (RZ) with Neutral Red staining dye and 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 Macrobella decora by an enzymatic technique (Sawada et al., J. Phys. Exp. Therap. 186:506, 1973) which is sensitive to 50 femtomoles of serotonin (5X10^-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 pmoles. 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 electrophysiological states is 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 genital ganglia was observed after bag cell excitation in vivo. 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 hemaphroditic duct caudal to the spermathecal duct). Homogenates of the atrial gland were able to release eggs from isolated reproductive tissues, 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 transitions in some cells were characteristic of 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 (JTL), NS05830 (JTW), NS07613 (JEB), and NSF grant PCM76-18936.


Sensory interneurons in the cricket receive a highly specific set of synaptic inputs from cercal sensory receptors. Both the receptive fields and the specific information which is processed by each identified interneuron are not fixed for life but are subject to modification by experience. The receptive field properties of the lateral giant interneuron (LGI) upon stimulation of the contralateral cercal receptors can be altered by a variety of experience-dependent and experience-independent mechanisms.

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 normal balance in synaptic inputs is essential for the proper development of synaptic specificity.

Supported by N.S.F. research grant No. BNS-7523454 to R.K. Murphy.
The effect of induced activity in central gill motor neurons L7, L9, and L7 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 depolarization to produce 1-5 AP's/sec and one series with L9 free running. In most preparations L7 or L9 was also recorded and depolarized on a 1-3 series. The habituation series were repeated at 15-30 min intervals for a period of at least 3 hrs. The intensity of the tactile stimulus was 1 gm. The following results were obtained with L7 and L9 depolarization: 1) induced activity in L7 resulted in an increased gill reflex amplitude but had little, if any effect on the rate or degree of gill reflex habituation or the synaptic decrement associated with it. The following results were obtained with L7 depolarization: 1) induced activity in L7 also affected gill reflex amplitude even though not to the same extent as did L7 or L9: 2) induced activity altered 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 L7 or L9 continued to decrease 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, L7, and L9 are probably in the periphery and not in the PAG. L9 may be part of the CNS control system which exerts both facilitatory and suppressive control over the PAG and gill reflex behaviors.

Supported by the Medical Research Council of Canada.

619 INHIBITORY RESPONSES EVOKED BY CHOLINERGIC AGONISTS IN CRUSTACEAN STOMATOGASTRIC GANGLION NEURONS. Eze Narder and Daniele Paupardin-Tritsch* . Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris 75005 France.

In order to use pharmacological agents as tools in neural circuit identification in the stomatogastric ganglion and at other Arthropod central synaptic connections, we have undertaken to characterize the pharmacological properties of various cholinergic agonists of the cells of the stomatogastric ganglion of the crab, Cancer pagurus.

Cells of the stomatogastric ganglion were penetrated with double barrel microelectrodes, and agonists were applied iontophorically 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 produced a two-fold shift to a more hyperpolarizing reversal potential (~170mV) in the depolarizing direction. The inhibitory responses were not blocked in 20 mM CoCl2, or 0 Ca++, high Mg++, thus showing that these responses were not synaptically mediated. Preliminary pharmacological studies have shown that high concentrations of both the agonists and mecholyl chloride, which indicate that these inhibitors are relatively ineffective in blocking these responses, possibly because of inaccessibility, or because the receptors are insensitive to technical artifacts.

On occasion MeCh biphasic responses were found at neuropile sites at which ACh applications elicited predominantly depolarizing responses. The current hypothesis is that ACh applications activate a ubiquitous extrajunctional, nicotinic depolarizing receptor, and that relatively non-selective, postsynaptic, ionotropic L-type calcium currents.

E.M. is a postdoctoral fellow of the Helen Hay Whitney Foundation.

The sensory system of the cricket, Acheta domesticus, develops extensively during the organism's postembryonic period of maturation. The majority of the adult complement of central-sensory neurons differentiates during this time. Sensory deprivation for the first four instars results in a progressive decrease in the responsiveness of the identified interneurons, 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 cereal sensory system in which it is sensitive to a reduction in neuronal activity.

We have attempted to characterize the transmitter mediating these connections by combined chemical and pharmacological experimentation. Microchemical assays for various putative transmitters (SHT, DA, histamine, 5HIAA and OCT) indicate that these substances are not present in the A neurons. The A-cell content of various amino acids (ser, glu, asp, tyr, gly) are in no way different from levels found in other pigmented Aplysia neurons.

From the present data and the evidence for the presence of the MGI, we conclude that the MGI is involved in the organization of the cellular array within which the A-cell is embedded. We have attempted to characterize the transmitter mediating these connections by combined chemical and pharmacological experimentation. Microchemical assays for various putative transmitters (SHT, DA, histamine, 5HIAA and OCT) indicate that these substances are not present in the A neurons. The A-cell content of various amino acids (ser, glu, asp, tyr, gly) are in no way different from levels found in other pigmented Aplysia neurons.

The relationship between sensory neurons and central nervous system is unknown but there remains the possibility of a direct connection. The results of our experiments support this view. 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.)


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 anlined in two similar behaviors, feeding and rejection. A stereotypic rejection sequence, which may last 20-30 seconds or even longer, always involves both buccal and proboscis movements. The participation in these movements of the buccal mass of Pleurobranchaea is described. The rejection response is, in every instance, a monosynaptic polarization involving an increased conductance mainly to Na+ . The monosynaptic polarization involving an increased conductance mainly to Na+ has been previously described by Fredman and Jahan-Parwar, (Br. J. Pharmacol. 57: 15, 1975) who described connections between the neurons of the A cell clusters (presynaptic) and the B cell clusters (postsynaptic) in the cerebral ganglia. 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.

Motor programs for feeding and rejection are similar, as might be predicted from the resemblance of the two behaviors. The relationship between the phases 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. These results support the hypothesis that a single pattern generator is capable of conserving 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.

(Submitted by N.I.H. (PHS) training grant GM-01990 to author, and NSF grant BNS 76-81233 to G. Mitros, Dept. Anat.)


Two bilaterally symmetrical clusters of neurons in the cerebral ganglion of Aplysia californica have been identified as projection neurons to follower cells in the abdominal, pedal, pleural and cerebral ganglia. The follower cell response is, in every instance, a monosynaptic EPSP which is not enhanced by application of quisqualic acid (1O-4M) desensitizes and completely blocks the glutamate-evoked response while even at 1O-3M it has no effect on the EPSP, and b) bath perfusion of quisqualic acid 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.)


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 [3H]tyramine in Manduca central nervous tissue in vitro. Structures were removed from pharate adults, incubated for 1 hr in 50 µl of modified Grace's insect tissue culture medium containing 94 µM [3H]tyramine. After incubation metabolites were extracted from the tissues and separated by high-voltage electrophoresis at pH L9. Three major metabolites of [3H]tyramine were detected. One has been identified as N-acetyltyramine on the basis of the following evidence: (1) its Rf value in a solvent system is the same as that of authentic N-acetylt yramine; (2) its mobility on paper in 4 different solvent systems and when electrophoresed at pH L9; and (3, 5, 6, 8) the compound is hydrolyzed to tyramine under the conditions of pH and temperature that convert standard N-acetylt yramine to tyramine; and (3) the compound and standard N-acetylt yramine are converted to tyramine by an N-acetyl amino acid hydrolase.

The second metabolite has several chemical properties compatible with its identification as tyramine-d-sulfate. Incubation of tissue with both [3H]tyramine and SO4 resulted in incorporation of tyramine and SO4 into the metabolite in nearly equimolar amounts. This metabolite has no net charge at pH L9. When exposed to the membrane at pH L10.0, it does not penetrate the membrane. This behavior is consistent with that reported for sulfated metabolites of monoamines from the lobster (Kennedy, J. Neurochem., 30:315, 1977).

The third metabolite is positively charged at pH L9. and exhibits electrophoretic and hydrolav behavior consistent with that of 3,5-dihydroxyphenylglycine, an N-acetylt yramine degradation product. The monosynaptic EPSP recorded from the cerebral ganglia of the Aplysia when the 3,5-dihydroxyphenylglycine found in lobsters (Kennedy, J. Neurochem., 30:315, 1977) is the role of the 3,5-dihydroxyphenylglycine found in lobsters (Kennedy, J. Neurochem., 30:315, 1977) in neurotransmission. Microchemical assays for various putative transmitters (SHT, DA, histamine, 5HIAA and OCT) indicate that these substances are not present in the A neurons. The A-cell content of various amino acids (glu, asp, tyr, gly) are in no way different from levels found in other pigmented Aplysia neurons.

The possible relationship of the tyramine-d-sulfate metabolite to the neurotransmission of the Aplysia is suggested by its chemical properties and by the recent demonstration of the presence of this compound in the cereal sensory system of the cricket (Matsumoto and Murphey, 1977, J. Physiol. 268: 533-548).
PARALLEL PROCESSING IN THE CRAYFISH OCCLUDOMOTOR SYSTEM: DATA AND POSTEMBRYONIC DEVELOPMENT OF AN IDENTIFIED INTERNEURON IN THE SOCIETY FOR NEUROSCIENCE

...potentials also exhibited faster rise times. In some preparations, augmented the psp produced by standard stimuli and blocked action...directly sensitive, its response increased monotonically with increasing stimulus intensities, and it received its primary excitatory input from the contralateral cec...potentials giving rise to the compound postsynaptic potential (psp) which occurs upon acoustic stimulation could be distinguished. Finer...rise times in the immature specimen than in the adult. Synaptic activity in the afferents which excite the MGI was lowered (sensory deactivation) at low intensity. These results suggest that the soma recording site is a better "window" into neuron function in the immature specimen than 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.


The function of the blue-sensitive receptors in the crayfish eye is the question of whether crustacean motion-detection systems are color blind, as insect systems are. We previously showed that the blue receptors contribute to the optokinetic tracking system.

Restrainted 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. The illuminating light path to vary the stripe radiance (range: 2 - 100 nW/cm²), and the corresponding amplitude of optokinetic tracking was monitored continuously with a calibrated radiometer. In 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, our experiments on different light positions were repeated at red or blue adapting lights flooding the inside of the drum. The normalized blue-adapted spectrum is the same as the mean spectrum of crayfish eyes 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. Biological. Biol. 47, 77-90 and 91-106, 1978). Thus they provide a readily available electro-physiological assay for peripheral neural regeneration and functional reinnervation of target cells. The culture technique involves expounding the paired buccal ganglia, the muscular buccal mass, and the salivary glands of one snail and implanting this neuroeffector complex in the hemocoel 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 neu...
629 CRAYFISH GIANT FIBERS ARE COMMAND AND DECISION NEURONS. Gene C. Olson* and Franklin R. Cray, 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 would 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 conveyor 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 during weight supported shock trials when 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. We conclude that the LGs do appear to be both command and decision neurons. (Supported by USPHS Grant NS08108.)


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 antenna-like cerci. Electronmicrographs of one of the giant interneurons, the median 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 amine containing 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 amine containing 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 afferent amine containing 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. Isolated perfusion of octopamine (10^-3-10^-4M) produces an increase of 10 to 20% in the response of the MGI to sound. The following amines are without effect at 10^-4M (sodium insensitive mechanism with K_m value of 1.4 x 10^-4M). The influx of tryptamine into small 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 K_m value of 8.5 x 10^-9M (sodium sensitive component) and a low affinity mechanism with a K_m value of 1.8 x 10^-5M (sodium insensitive component).

Further evidence that the tryptamine influx mechanism is similar in character to the low affinity uptake system for 5-HT, i.e. sodium insensitive with relatively high K_m value. The influx of octopamine is thus, in principle, further evidence that octopamine is a modulator of central nervous functions, a relationship which has not been previously considered.

Supported by: Cellular Biology Section, University of Southern California (M.O.) and NSF Grant 80MS5723456402 (R.K.M.)
EFFERENT INFLUENCE OF THE CEREBRAL GANGLION ON PHASE SETTING OF L = 4.17 ± 1.04 s.; PS = 0.41 ± 0.04 s. P = 4.87 ± 1.03 s. 

Variation in PS latency (L-L), Fig.1, and variation in PS duration (PS-PS), Fig.2, correlated with variation in period (P-P). 

EFFECTIVE INFLUENCE OF THE CEREBRAL GANGLION ON PHASE SETTING OF THE CIRCADIAN OSCILLATOR IN THE APlysia EYE. ROBERT G. PRICHARD* AND RICHARD L. LICKLEY. Dept. of Zoology, Oregon State University, Corvallis, OR 97331. 21 h of LL. This shows that the temporal organization of this central program shares 2 salient features with both the behavior (2) and motor pattern no.1 (3); the post-cellular relations of L, G, and period of return-stroke neuron bursts (RS) have similar slopes (Fig.1:1:2) and PS durations are nearly invariant (Fig.2:1:2). The central motor program that emerges 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 include the longer duration of PS 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. & verr. Physiol., 75, 233-258, 1971.

A CENTRAL MOTOR PROGRAM FOR UROPOD BEATING IN THE ANOMURAN CRAB, Emirita analoga. DOROTHY G. PAUL. Dept. of Zoology, Oregon State University, Corvallis, OR 97331.

Two rhythmic motor patterns in the same locomotors underly beating of the pair of swimming appendages (uropods) in the sand crab, Emirita analoga. One motor pattern, no.2, associated with treading water, generation of bursts in the power-stroke motor neuron (PS) is dependent on proprioceptive feedback from return-stroke (RS) C11 ganglionic motoneurones. The second motor pattern, 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 and motor pattern no.1 (3); the positive correlations of L, G, and PS durations are nearly invariant (Fig.2:1:2). The central motor program that emerges 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 include the longer duration of PS 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. & verr. Physiol., 75, 233-258, 1971.


In an Aplysia monosynaptic pathway, imposed hyperpolarization of the pre-CTT 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" of 0.2mV (PSP/mV) (soma potential). At 10mV (resting), PSP amplitude was close to maximal. Polarization for less than 50mV, 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 -50mV initiated release and was sustained for at least 2 sec; electrotactonic coupling between IN and LGC was excluded with TTX. We conclude that some polarization controls release at the terminals. Control probably is by electrotactonic spread to the terminals and involves Ca: Increased Ca++, 33mM, partially protected release from hyperpolarization and enhanced the effect of depolarization to decrease both Ca diffusion and to accelerate Ca movement towards the terminals. This post-tetanic potentiation, characteristic of this synapse, was enhanced by some depolarization and suppressed by hyperpolarization Of the electrical function 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, INNUM.)


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 (motor neuron fig. la, lcb), inhibited by TTX, but not with TTX in bhr. Bath application of TTX sufficient to block spikes also halts spontaneous cycling. Bath applied TTX plus 1mM Dopamine induces patterned cyclic activity without action potentials (motor neuron fig. la, lcb), localized toxin blocks LP in bhr. Induction involves the activation of endogenous bursting properties in the AP, 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.) The assistance of T. D. Barker have made similar observations independently.

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 92037.

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 (motor neuron fig. la, lcb), inhibited by TTX, but not with TTX in bhr. Bath application of TTX sufficient to block spikes also halts spontaneous cycling. Bath applied TTX plus 1mM Dopamine induces patterned cyclic activity without action potentials (motor neuron fig. la, lcb), localized toxin blocks LP in bhr. Induction involves the activation of endogenous bursting properties in the AP, 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.
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, CaCO3, vitamins in agar matrix) can be recorded with 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) can be recorded with an artificial diet. 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 buccal masseteric organ. Intracellular electrodes record sensory input from the buccal complex and motor output underlying feeding movements. Artificial foods added to buccal complex structures such preparations allow us to examine 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 5F32NS05188 and NSF grant BNS 76-18792

MOLT-RELATED "NEUROSECRETORY" CELLS IN THE ABDOMINAL NERVE CORD OF THE CRAYFISH, PROCAMBARUS CLARKII. Richard L. Roth* (SPON: Jeffrey J. Wine) Dept. of Biology, Stanford University, Stanford, CA 94305.

Near the central margin of each thoracic and abdominal ganglion of the crayfish, Procambarus clarkii, there are two pairs of somata (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 the other 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 Giemsa'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 depletions are 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 coelom backfills that these apparent neurosecretory cells may also serve as ascending interneurons. Efforts to confirm this suggestion by intracellular recording with microfilled microelectrodes are now in progress. It is also of some interest that a brief transition from aggresive to escape behavior occurs in conjunction with the discharge of the fuchsinophilic product. It is possible that the shift is causally related to the neurosecretory activity is under investigation.

Supported by NIH Grant NS 02944 to D. Kennedy.


Seven different muscles in the walking leg of the scorpion (Panuroctonus nesaemis) 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 1977). Compared to crustacean and insect muscles, scorpion leg muscle fibers are strikingly uniform in sarcomere length both within and between animals and within and between muscles. The fibers are small (15-30 microns in diameter) and are closely packed within the muscle. The fibers consist of a central region of nuclear material 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 muscle also includes a peripheral area, thin-thick filament ratios, banding patterns and synaptic ultrastructure. Intracellular recording together with light microscopy resolves the details of each synaptic transmission and, innervation patterns for the entire leg. Values were obtained for resting potential, input resistance, number of motor nerve fibers, and various parameters of the muscle fibers. Features such as facilitation and fatigue are also described.

Supported by USPHS grant NS 14555 (M.R.) and NIH grant HL 22902 (R. F. B.)

Aplysia californica release ink in response to strong noxious stimuli. Ink releasing 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 in ink release alone, a steep input-output relationship was measured as a function of stimulus duration.

Three types of behavioral experiment demonstrated that ink release is selectively sensitive to long duration 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 animal was 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 L14 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 electrostimual 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 acceleration of L14 spike activity is responsible for the five sec max. stimulus is due to an increase in synaptic 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 synapt current. 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.

RECEPTOR SOURCES OF EGG-LAYING INDUCTION ALSO INFLUENCE THE ISOLATED APLYSIA HEART. T. Smokt; S. Arch; and P. Lloyd (SPD, R. Rhodes). Biological ganglia are 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 revealed that one of the peptide fractions isolated from the atrial gland was active on the heart. Neural crest cells in the connective supplying 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 acceleration of L14 spike activity is responsible for the five sec max. stimulus is due to an increase in synaptic 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.

SYPNCTIC PHYSIOLOGY AND PHOTOACOLOGY OF A WHITE, PUTATIVE COMMUNICATING INTERNEURON (C2) IN TRITONIA DIOEDEA. Robert Snow. Dep. 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. The activity is apparently required for the initiation of each swim cycle (Getting, J. Comp. Physiol. 121: 325, 1977; Taggart and Willows, J. Comp. Physiol. 123: 253, 1975). Antidiromic stimulation of all of the brain roots suggests that C2 is an interneuron having no peripheral directed processes. C2 make contralateral synaptic connections with all cells and has been shown to mediate the cardioaccelerating effects of many pharmacological agents, including atrial natriuretic peptide (ANP) and angiotensin (Ang II). C2 is a monosynaptic connection with the ventricle. Afferent input to C2 is the afferent from the para-vascular organ which is thought to excite C2. C2 may also receive input from C1, a cholinergic neuron. The activity of C2 is monitored in small rectangular glass chambers (55mm x 15mm x 5mm) fabricated from micro slides. In the light, C2 is active during the dark. When placed in constant light, LL activity occurs in bursts of about 1 hr separated by 2 hr 'rest' periods. This behavior persists throughout the day with no circadian component. No tidal or circadian rhythms in the behavior of Plama are evident. Supported by NIH NS 05423.

SYNECTIC PHYSIOLOGY AND PHARMACOLOGY OF A WHITE, PUTATIVE COMMUNICATING INTERNEURON (C2) IN TRITONIA DIOEDEA. Robert Snow. Dep. 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. The activity is apparently required for the initiation of each swim cycle (Getting, J. Comp. Physiol. 121: 325, 1977; Taggart and Willows, J. Comp. Physiol. 123: 253, 1975). Antidiromic stimulation of all of the brain roots suggests that C2 is an interneuron having no peripheral directed processes. C2 make contralateral synaptic connections with all cells and has been shown to mediate the cardioaccelerating effects of many pharmacological agents, including atrial natriuretic peptide (ANP) and angiotensin (Ang II). C2 is a monosynaptic connection with the ventricle. Afferent input to C2 is the afferent from the para-vascular organ which is thought to excite C2. C2 may also receive input from C1, a cholinergic neuron. The activity of C2 is monitored in small rectangular glass chambers (55mm x 15mm x 5mm) fabricated from micro slides. In the light, C2 is active during the dark. When placed in constant light, LL activity occurs in bursts of about 1 hr separated by 2 hr 'rest' periods. This behavior persists throughout the day with no circadian component. No tidal or circadian rhythms in the behavior of Plama are evident. Supported by NIH NS 05423.

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 a previously unreported cell line of glial-like cells, since Coggeshall (J. Neurophysiol. 30:1263, 1967) presented evidence that their numbers approximately triple between juvenile (5 g, body weight) and adult (110 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 Essential medium made up in FSW. This medium has been shown to support dissociation of cells. We estimate about a 30-50% recovery during dissociation of the bag cells. We also obtain glial and other unidentified cells.

After removal of the BC clusters from the abdominal ganglion, the connective tissue capsule is removed and the bag cells are disaggregated using a Sliicard-pasteur pipette. 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 connective tissue capsule, and the numbers approximately triple between juvenile (5 g, body weight) and adult (110 g).

Many of the BCs and other cells attach to the plastic surface of the dish (Falcon, 35 mm) and develop neurites, within 24 h, 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 the BCs exhibit many of the same properties as a vertebrate neuron, body indicating that a heteropolar neuronal condition can be expressed, at least in tissue culture, and is not unique to vertebrate neurons.

The intracellular recordings from BC neurones in primary cell cultures 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 an 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 input resistance of ~60 μm diameter BCs (without neurites) are approximately 30,000 Ω.cm² and 0.5 μS/cm. The surface receptors of BC neurones are being explored with iontophoretic applications of various transmitter analogues. We find that many BCs respond to serotonin (corticost III) with depolarization and in those BCs with APs serotonin may cause firing.

Plasticity of feeding behavior in the opisthobranch mollusc Navanax inermis. A.J. Suswein*and M.V.L. Bennett (School of Science, Division of Health Neurobiology, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y.10461).

Plasticity of feeding behavior was investigated in Navanax. Navanax is a carnivore which ingests prey with suction caused by rapid pharyngeal expansion. Swallowing movements move the prey into the esophagus. Feeding is little affected by handling or posture, and is more resistant to noxious stimuli than in other gastropods. By contrast, ingestion is readily affected by recent feeding history. 1) Arousal. Feeding latency is decreased for feeding experiments when specific site or ingestion of ~60 μm diameter BCs (without neurites) are approximately 30,000 Ω.cm² and 0.5 μS/cm. The surface receptors of BC neurones are being explored with iontophoretic applications of various transmitter analogues. We find that many BCs respond to serotonin (corticost III) with depolarization and in those BCs with APs serotonin may cause firing.

PRESYNAPTIC ACTION OF GLUTAMATE AT A CRAYFISH NEUROMUSCULAR JUNCTION. H. Thieffry*and J. Bruner*(SPON: I. Kupfermann). Division of Cellular Neurobiology, Université de Picardie, 80039 - Amiens, France.

It is largely admitted that glutamate depolarizes crustacean muscle fibers by acting on muscle membrane receptors. However, the exact way glutamate acts on the muscle membrane has not yet been determined. The idea that "acute denervation" induced by isotoic concentration of Ca strongly depressed the glutamate response of fast abdominal ganglion of crayfish Procambarus clarkii to this observation which is in favour of a presynaptic action of glutamate, previously suggested by other authors with further support 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 750 μm from the site of intraxonal recordings.

Both application of glutamate (0.1mM) depolarized both the muscle fiber and the axon by about 20 mV. Glutamate iontophoretically applied to the axon far enough from the muscle fiber induced the response in the axon thus implying the existence of glutamate receptors on both muscle fiber and the axon. 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 nerve 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 N.I.S.E.R. grant n° 287670).

THE responses of cerebral receptors and identified interneurons in the cricket (Acheta domestica) to air streams. Martha Tobias* (Sponsor: 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 simple 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 body. 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 elicited 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 toward 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 75-3345 A01 to R.K. Murphy.

SPONTANEOUSLY active molluscan neurons: spike initiation zone lies in axon. Steward A. Trelitman. 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 axonal cells were isolated by either ligation or enzymatic dissociation from their axons, and still exhibited patterned spike output. By recording simultaneously from cell body and axon of intact pacemaker neurons RIS, 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 BNS 77-01548.)

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 125I-α-bungarotoxin to membrane fragments prepared from Limulus brain tissue has been reported (Thomas, et al., Arch. Biochem. Biophys. 187:53). The kinetics 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 10-5M d-tubocurarine, nicotine, scopolamine and pilocarpine, respectively. Phenylmethylsulfonylfluoride and ethylenediaminetetraacetic acid inhibited the solubilized extract 72.1%. TEA was used to eliminate neuron action potential from a half-width of 15-30 msec to one of 62.5-125-300 msec. In contrast, all other drugs produced either no increase or only insignificant small increases (generally less than 50%) in spike width.

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 insignificant 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 neurotransmitter. 5-HT, however, since more exciting agents 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.

I have 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 NSNS05617.
653 IDENTIFICATION OF MOTOR NEURONS MEDIATING OPALINE SECRETION IN APLYSIA CALIFORNIENSIS. Susan Tritt*. and John H. Byrne (SR09). O. Framm Dept. of Physiol., School of Medicine, University of Pittsburgh, Pittsburgh, Pa., 15261.

Response to noxious stimuli Aplysia californiensis characteristically releases two substances, ink and opaline, which are believed to act as defensive mechanisms against potential predators (Bales, 1921). Whereas there is now a fairly good understanding of the control of ink secretion, we have no way of making a large contribution to the motor component of this behavior.

Electrical stimulation of tegumentary nerve 7 which innervates the region of the pedal ganglion, results in a rapid marked contraction of the opaline gland itself. When the motor neurons in the abdominal ganglion which mediate inking behavior, the pleural ganglion cells released their secretions which were of the same intensity as those produced by electrical stimulation to the pedal ganglion.

Electrical stimulation of the pedal ganglion releases a secretion into the coelomic fluid of the right pleural ganglion, which is a continuous, continuous stream throughout the body. The secretion is characterized by a high viscosity and contains a large number of opaque, elongated bodies, each of which is 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 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 this behavior, the pleural ganglion cells have relatively high resting potentials and are normally silent with occasional background subthreshold EPSPs. Another similarity between the motor neurons which mediate opaline secretion is that the opaline motor neurons are electrically coupled with a coupling ratio of about 0.3. A train of electrical stimulation to the right neural-abdominal inhibitory neurons produces a large summating EPSPs in the opaline gland motor neurons which cause the cells to fire and lead to contraction of the opaline gland.

Intracellular recording from the pleural ganglion cells, demonstrates a range of firing rates up to 90%. Similarly firing individual neurons with intracellular electrodes, applied to the pleural ganglion cells, results in an EPSP with a decay time constant of 55 ms and a firing frequency of 20 Hz.

The results indicate that an identical "conflict" stimulus to the right pleural ganglion affects the opaline motor neurons to produce a change in the response pattern of the pedal ganglion cells. 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 noxious stimuli. Supported by NIH grant NS18151 and NS10889.


Our investigation of the nervous system of the nematode Ascaris lumbricoides has employed a combination of anatomical and physiological techniques. The shape of neuron of which the branches have been reconstructed from serial 10 pm sections in the light microscope. Criteria for identifying synapses in the light microscope have been developed and are outlined by examining selected sections of the sympathetic neuropil in the light microscope. The neurons are divided into seven classes, based solely upon shape (Stretton et al., 1978, Am. J. Anat., 153, 317-340). Synapses in the dorsal cord, the excitatory motorneurons (DE1, DE2, and DE3) synapse directly onto the ventral inhibitory motor-neurons (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 with other motorneurons.

The synapses between the dorsal excitors and the ventral inhibitory dendrites have been investigated electrophysiologically. Activation of a single motor neuron and recording intracellularly from a muscle along the length of the dorsal cord, the conduction velocities of each of the dorsal cord excitors have been found to be 25 cm/sec. The muscles send as much of the nerve fibers as are available to another in a region called a "sycnaptic". This arrangement permits the conduct of the action potential through the synapses. Recordings taken from the muscle fibers show that the potential is conducted in the dorsal cord, the demonstration of a conduction velocity of 12 cm/sec in the muscles. Since a wave of propagation moves along the body of the motor neuron at 10 cm/sec, we can rule out the simple model in which the velocity of propagation of an action potential along each excitatory motor neuron or the sycnaptic cor responds to the velocity of propagation of a body wave. (Supported by PHS Grant NS11006, NSF Grant 76-13961, Sloan Foundation, and The Research Fund of the Graduate School, Madison)


Locomotion in Aplysia californiensis is a complex centrally coordinated motor sequence (Bisbing et al., 1977) which is modulated by a variety of internal and external stimuli (Kupfermann, 1968; Advokat et al., 1977); Heiss et al., 1977). We here explore the locomotor consequences of conditions which either facilitate or depress this motor program on response selection in a subsequent "conflict" situation.

Antraxia 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 studied the effect of the noxious stimulation on the immediate selection of a defensive program. In all experiments, walking was measured using a blind procedure. We found animals receiving startling 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 580 sec, p<.001). The head-group also walked more slowly than the tail-group (p<.01). Similar results have previously been described by Kandel (1973) and Impallari and Kandel (1977) that finding elicted by salt to a parapodium can be terminated by a noxious stimulus to the head region.

The present 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 shock 8 sec applied alternately to the head and tail for 20 sec. In response to the "conflict" stimulus control animals with no previous shock (N=10) showed a latency 40 sec. By contrast, animals which previously received tail shock walked significantly faster (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<.02 respectively).

These results indicate that an identical "conflict" stimulus can produce opposite effects on escape walking depending on the animal's past experience. Unlike stimulation 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 responses to a particular stimulus.

656 INTERNEURONS MEDIATING SPATIAL POSITION IN A COCKROACH. W. William Walthall and N. Bernard Hartman. Dept. of Biol. Sci., Texas Tech University, Lubbock, TX 79409.

The equilibrium receptor system for the cockroach Amelesana consists of four interneurons, each excited by primary sensory neurons from one of four rows of tricholaths. Tricholaths are sensilla sensilla suspended from the ventral basal region of the cerci, specifically adapted to perceive information regarding the insect's spatial orientation.

Interneurons mediating spatial position are inactive when the insect is stationary. Displacements from that position evoke responses from one or two of the interneurons. These interneurons fire tonically to maintained displacement without change in orientation. In each instance, the frequency of firing and the rate of adaptation is proportional to the angular displacement. Physiological evidence indicates that each interneuron independently responds to rotation in a different quadrant. Discharge from two interneurons occurs in regions where adjacent quadrants overlap, these being roll right and left, 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.

Amelesana 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 fixed orientation. The cockroach equilibrium receptor system of the cerci is a particularly desirable adaptation. The employment of giant fibers to mediate this utility suggests that this insect has made a considerable investment in modulating locomotory behavior. Our physiological 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 NSF-172283 and National Aeronautics and Space Administration grant NAGS-7435.

INVERTEBRATE NEUROBIOLOGY
modulatory type involving long duration of action and voltage-sensitive ionic conductances. Previous data suggested that the synaptic potential comprising an input to the MCC and the synaptic current are currently studying the ionic basis of the synaptic current. The implication is that these interneurons are electrically coupled to certain of the motor neurons, and in a few cases, connected by interneuronal stimulation whilst the frequency of the bursts in these interneurons may not be essential to driving the entire system. Brief (less than 2 s.) stimulation of particular neurons in 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 different from two other mechanisms of visual origin which allow for discrimination of small field stimuli: (1) Feed-forward inhibition to the LGNd posterior to the site of convergence of the efferent pathway, produced by large field stimuli and (2) lateral inhibition presynaptic to the dendritic arborization of the LGNd which suppresses large field stimuli and reduces habituation at the excitatory afferent-LGNd synapses (O'Shea and Bowler, Nature 254:53, 1975; Bowler, R. O'Shea and Williams, J. Exp. Biol. 86:157, 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 movement detector system.

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

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.


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, E15+22) and neonatal (The day of birth and postnatal day 1, P1+2, P1+21) periods. The average age of labeling was 4 h after an injection of 3H-thymidine added during each day of formation were determined at several anatomical levels within each septal region nucleus. There were significantly different wavefronts of neurogenesis (and between nuclei). The neurons of the midline nuclear group (diagonal band, medial, and triangular septal nuclei) formed between E12-13, the lateral septal nuclei (LSe) between E14-15, and the nuclei of the stria terminalis and anterior commissure between E14-18, the nucleus accumens 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 nuclei, forming 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 cellular components, one represented by the homogenous neuroepithelium and the other represented 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.
EFFECTS OF SEPTAL LESIONS ON THE RENAL SODIUM GRADIENT. Wail A. Bengeloum, Khadija Badouri* and Mohamed El Hilaïfi*, Dept. of Biology, 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 the serum sodium concentration was increased and urine output was decreased following septal lesions in rats (2,3). In the present study we attempted to establish whether this was also the case for renal and urine output. Na concentrations were determined by flame photometry, whereas ADH replacement eliminates septal lesion-induced polydipsia and polyuria (5). This latter finding suggests a lesion-induced decrease in Na secretion (5), and such a phenomenon would be consistent with the renal cortico-medullary Na concentration gradient (7).

Water intake of sham-operated and sham-operated rats were monitored starting at 1 wk prior to surgery and continued for 2 wk 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 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. Similar Na concentrations in the renal cortex and external medulla of septal rats did not differ from control values. In the combined renal internal medulla and papilla, however, there was 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 provide 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 septal region in water intake.


It has been clearly demonstrated that trigeminal somatosensory input from the region of the trigeminal nucleus constitutes an important element for the occurrence 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 amygdaloïd 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) hypothalamic-evoked quiet biting attack. In stage I of the experiment, the lateral extent of the lipline that, when probed, could elicit the jaw opening response was determined. Other observations suggest that the amygdala does not significantly inhibit quiet attack also significantly reduced (p<0.01) the lateral extent of the lipline from which jaw opening could be elicited. When tracing the lateral extent of the lipline, the lateral extent of the efferent projections of the amygdaloid complex was determined. The effect of electrical stimulation of the amygdala upon the "effective" extent of the lipline was determined. The results indicate that sites in the amygdala which significantly increased (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 (p>0.1) the lateral extent of the lipline from which jaw opening could be generated. 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<0.01). 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)
HIPPOCAMPAL INFLUENCE ON AFFECTIVE COMPONENTS OF FEMININE SEXUAL AND AGGRESSIVE BEHAVIOR IN THE RAT: DORSAL-VENTRAL 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 the limbic structures (i.e., sepiatal 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 (DEL) or posteroventral (VHL) aspects of the hippocampal formation of ovariectomized rats. Sham-operated 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. DEL 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 DEL 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, with 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 inputs into the hippocampal structures mediating feminine sexual and aggressive behavior. This input may best be characterized as mediating an affective component of feminine sexual and aggressive behavior.


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 (0.02-0.05µ) 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 perfused with dithioKarovsky’s fixative in phosphate buffer (pH 7.4). They were sectioned at 80µ, and reacted with DAB. Examination of these sections revealed numerous NRP 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 the hippocampus (as recordings from hippocampus and entorhinal cortex project outside the hippocampus). The NRP technique does not allow statements to be made of the terminations of these efferent but the involved neurons clearly cannot be considered interneurons or short-axonated cells. Supported in part by Intramural Research Grant from the College of Medicine.


Previous investigations have demonstrated an early, rapid and substantial increase in hippocampal neuronal activity induced by a behavioral learning paradigm. While certain anatomical- and electrophysiological evidence suggests the entorhinal-dentate synaptic system may be a potential site of facilitation, the possibility remains 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 hypothetically distinct neuronal systems.

Microelectrodes were implanted in the CAI 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 US 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 trials, recordings from hippocampus and entorhinal cortex proved markedly different across trials (that is, over the course of learning). Within trials, neuronal discharges from both structures exhibited increases in both latency and discharge rate (compared to spontaneous rates) during the UCS period early in training—prior to behavioral conditioning. Unit discharges were also modulated by shock in both structures during the CS period as behavioral conditioning developed. Across trials, however, entorhinal discharges remained constant or increased slightly, yet rapid and substantial increases occurred in the hippocampal discharge. Such hippocampal neuronal increases cannot be accounted for solely on the basis of activity either in entorhinal cortex or, as previously noted, in medial thalamus (the major afferent to the hippocampus). Thus, these findings suggest facilitation across the entorhinal-dentate synapse and indicate the initial locus for generating learning dependent neural changes to be the hippocampus per se.


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 lesion, thus producing a degenerating pattern which combines fibers of passage and the degenerating system of interest. This problem can be circumvented through the use of radioautographic tracers, using labelled amino acids and providing information about terminal fields and cells of origin. These methods do not, however, allow detailed tracing 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 the use of kainic acid lesions in combination with Fink-Heimer silver staining of degenerating axons and terminals as 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, vibration sections, prepared for the detection of catecholamines, enable evaluation of the extent to which presynaptic dopamine islands remain undamaged following cell destruction by kainate. (Supported by NS 19388 to A. R.)

CHOLINERGIC MECHANISMS AND POST-TETANIC POTENTIATION. J.F. DeFrance, J.C. Stanley, J.E. Merchand, P. Divakaran and J. 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. The rabbits were acutely prepared under urethane or urethelose chloride 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 nuclear complex (MSN), (2) from the contralateral hippocampal field CA3 (CCA3). Stimulation of MSN and CCA3 was done with microelectrodes (1-50 megohms). The threshold for various drugs on the normal and potentiated responses, multi-barrel electrodes were used. This array included a recording barrel and a current-summing barrel in addition to the drug containing barrel. The compounds studied were: acetylcholine (ACH: 0.1-0.5M, pH 6.7), guanidine 3',5'-monophosphosphate (cGMP: 0.1-0.5M, pH 6.5), atropine sulfate (Atrop: 0.5-2.0 µM, pH 6.2), phentolamine maleate (PM: 0.5-10 µM, pH 6.5). (PM was replaced by methyl xanthine [MIX: 0.1M, pH 6.5], KCl (1.0M, pH 6.8). Theophylline was administered peripherally (2.0-6.0 mg/kg). ACH, phentolamine, and MIX 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 75-100% potentiated, whereas cGMP activated primarily postsynaptically for the enhancement of pyramidal cell excitability.

It was suggested, with respect to the potentiation phenomena, that ACH might act primarily presynaptically to facilitate transmitter release, whereas cGMP activated primarily postsynaptically for the enhancement of pyramidal cell excitability.

The electrophysiological 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 postsynaptic potentiation of stimuli which maximally potentiated was post-tetanic potentiation. Cyclic AMP levels were only slightly depressed. Supported by NSF GB35532 and NIH #F32 NS00874-01.

AUTORADIOGRAPHIC INVESTIGATION OF THE CENTRAL PROJECTIONS OF THE OLFAC'TORY TRACTS IN MACROPODUS OECOPHILICUS (L.) (OSTEICHTYES: BELONIDAE). Roger E. Davis, Mental Health Research Institute, University of Michigan, Ann Arbor, Ml 48109.

The distribution of the lateral and medial olfactory tracts was investigated using radioautography. Twenty-six adult male Macropodus were administered 0.2 to 0.5 µl of lH-2,3H 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 neorubric 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 bed, the medial and lateral subpallium, and the projections to the posterior thalamus and hypothalami. However, nucleus taenia and area Dp were only slightly labeled.


In a study on the psychophysics of self-stimulation a rewarding hypothalamic stimulation served as a conditioned stimulus (CS) in an avoidance paradigm in the rat. Generalization tests were conducted by modifying the electrical parameters of the CS forming a set of substitute stimuli (S.E.). Results show that a Steven's power function relates the reinforcing sensation, measured by such generalization tests, to the intensity of the rewarding stimulation (calculated by the bar-pressing rate for all the S.E.).

The effects of a concomitant painful footshock upon the rewarding hypotalamic stimulation were then studied. Data show that the greater the intensity of the footshock, the greater the length of each self-stimulation interval. Further studies showing that injection of naloxone (5 x 10^-6 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
680 GENETIC AND NEUROCHEMICAL ASPECTS OF THE ACQUISITION OF AVOIDANCE
681 BEHAVIOR. Herbert J. Doller* and Forrest F. Weight (SPON: A. P. Oliver). Laboratory of Neuropharmacology, NIMH; Saint Elizabeths Hospital, Washington, D.C. 20032.

682 A hippocampal pathway originating in the entorhinal cortex, traversing as part of the perforant path, and terminating in 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 Prlbrum (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 in 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.

683 ELECTROPHYSIOLOGICAL EVIDENCE FOR THE PERFORANT-CA1 PATHWAY IN THE
684 HIPPOCAMPAL SLICE. Herbert J. Doller* and Forrest F. Weight (SPON: A. P. Oliver). Laboratory of Neuropharmacology, NIMH; Saint Elizabeths Hospital, Washington, D.C. 20032.
HIPPOCAMPAL FIELD POTENTIALS AND SINGLE UNIT ACTIVITY EVOKED BY STIMULATION OF MEDIAL SEPTAL NUCLEUS, ENTORHINAL CORTEX AND recording neocortical EEG and neck-muscle EMG were also implanted trained to walk continuously on a motor-driven treadmill. They were housed in the treadmill for several days, so that after recovery, recordings could be 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 change over 600 min, indicating a stable system, and its characteristics were similar to the facilitation noted in the EC to fascia dentata system. 2) 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 V. E. Amassian.)

ATTENTION OF SEPTAL HYPERREACTIVITY BY INTRACRANIAL INJECTION OF MORPHINE INTO THE PERIAQUEDUCTAL GREY (PAG) Prod H. Guillory, 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 10 minutes. This increase in responsiveness to various stimuli has been described by others. We have further characterized and quantified this phenomenon which we have previously described as an increase in aggression, irritability, emotionality, or rage; the common element of the syndrome reflects an increase in unconditioned responding to various stimuli. We have previously demonstrated the possible involvement of both norepinephrine and serotonin in modulating this hyperreactivity. Opioid narcotics have a similar effect, and under appropriate conditions both norepinephrine and serotonin are involved in the modulation 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 iul injections of either saline or morphine (5µg/ul or 15µg/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, polygraph 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 iul injection of naloxone (40µg/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 and sensory abilities were impaired, and suggests the involvement of brain mechanisms of pain perception in the syndrome.


In Nissl stained material the intercalated masses (MI) are clusters of medium-sized (15-20) cells in the external capsule and longitudinal (L) and transverse (T) tracts suggest that the MI is lateral and basolateral amygdala. One large island appearing beneath the anterior commissure's lateral wing, is displaced ventromedially by the basolateral nucleus. Usually, it lies in the fibers of the stria terminalis (ST). The axons of the MI within the major fiber bundles of the amygdala prompted this analysis.

The appearance of the MI in Nissl material behaves its heterogeneity and the formidable extent of their processes. Intercalated cells within the external capsule and L and H have similar primary and secondary dendrites oriented parallel to the entering and exiting axons in frontal sections. The cells of the MI in the stria terminals are identical except the dendritic shafts in the to the stria terminals are often so dense that they obscure the dendritic tree. Individual axons are extremely short; as many as four separate peduncles and twigs arise from a single stalk. Somatodendritic axons are also observed. The axons give off several collateral cells 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 L and H. These axons give their branches to the extrinsic afferent systems which emerge from the soma and are oriented parallel to the L and H. The most striking feature is the length of the primarily unbranched dendrites, which are more than 1 mm long from tip to tip, form a formidable dorsal-ventral band between the basolateral and lateral nuclear and mediodorsal areas. The patterns of the axons, while in number, are orangetr and unusually long (10µ).

Many cells in the single large MI body are not way of passing axon systems appear as a mass or ganglion. The axons of the cells, while in number, are or many. The axons of these cells also enter several local collaterals. A single branch may branch to either the terminalis or one of the other septal nuclei.

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 view of the 60% MI cells give rise to large fiber 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 RO1 MH 28678.)


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 of reward, but only when reward was not obtainable. The performance of lesioned animals was also relatively unaffected by differences or shifts in amount of reward. In Experiment II, rats were tested in a progressive discrimination problem. They found food at the end of a black (white) straight-alley and no food in a white (black) alley. Amygdala-lesioned rats ran significantly faster to the food in the black alley than Controls in the positive alley, but significantly faster in the negative alley. These subjects were subsequently tested in a 2-tone, one of which was paired with primary reward and the other was not. There was no response to the food in the positive alley, but significantly less often than Controls. This suggests the amygdala is involved in situations involving the assignment of value to stimuli. In Experiment III, separate groups of subjects were exposed to 125 minute periods of low and high reward under conditions of reward. Controls increased their running speed as a function of reward amount. Rats with amygdala lesions, however, showed less overall anticipatory responding than Controls. 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 reward rats were left undisturbed while the other half were subjected to a 2-hour period of restraint. 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 reward rats were left undisturbed while the other half were subjected to a 2-hour period of restraint. 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 reward rats were left undisturbed while the other half were subjected to a 2-hour period of restraint. 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 reward rats were left undisturbed while the other half were subjected to a 2-hour period of restraint. 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 reward rats were left undisturbed while the other half were subjected to a 2-hour period of restraint.
CORTICAL PROJECTIONS OF THE THALAMIC MEDIODORSAL NUCLEUS IN THE OPOSSUM. Gregory T. Golden, Jan C. Jackson* and Robert M. Benjamini. 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.

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 0.1µl 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 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. Forty-eight hours after injection, massive invasion of the granular layer of the vermis was apparent around the injection site. Purkinje cells nearby appeared paler staining and gliosis was prominent.

Control injection of colchicine seems to produce the same 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 ROI NS12353 to O.S.)

SUPPRESSION OF EMOTIONAL BEHAVIOR IN CATS BY STIMULATION OF VENTRAL SEGMENTAL 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 concomitant electrical stimulation of the ventral tegmental area (VTA) or nucleus accumbens (NA) on lateral hypothalamic-induced attack behavior in cats. Low frequency (60ppa) stimulation of VTA or NA suppressed hypothalamically-induced attack without affecting the autonomic or non-directed somatic components of the attack reaction. High frequency (60ppa) 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 functional mesolimbic dopamine system is to inhibit emotional behavior.


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 injection sites. 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 at spatial memory are consistent with data from total hippocampus ablations, but those on emotionality are not.
DEMONSTRATION OF THE HABENULO-INTERPEDUNICAL FIBER SYSTEM BY 
\([1^4C]\)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-\([1^4C]\)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 anesthetized 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 anesthesia. Since anesthesia (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 anesthesia can selectively spare metabolism in an anatomically defined neuronal fiber system including the regions containing the cells of origin, the myelinated fiber and the terminal field. The actual subcellular components 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 consumes less glucose than does gray matter and 2) anesthesia generally reduces consumption in both gray and white matter. The greater glucose utilization by the tract than by the surrounding gray matter under conditions of anesthesia suggests that the habenulo-interpeduncular tract has unique metabolic and functional properties.

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.


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 behavioral areas: posterior CA1 cell field and alveus, 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 chalamic 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.


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 C).

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 hippocampal, and to a less extent in fimbrialis, was due to the animals re-entering 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.)
ATTENUATION OF LATERAL HYPOTHALMIC SELF-STIMULATION BY MICROINJECTION OF LIORESAL INTO THE VENTRAL ANTERIOR NUCLEUS OF THE THALAMUS. James A. Johnson and Ernest E. Kent. Dept. Psych., UICC, Chicago, IL 60680

Male Sprague-Dawley rats were implanted with 70-μm twisted wire stainless-steel microelectrodes directed at the far lateral aspect of the lateral hypothalamus (Pellegrino and Cushman coordinates: AP +5.4, L +2.1, I 1.8). In addition, 27 guage injection cannulas were back-filled bilaterally at the ventromedial nucleus of the thalamus (VAT).

The rats were trained to press for constant current, 8-mA, and were then trained to maintain stable current levels (usually 60 to 80 μA). Rats pressed at rates averaging 200 presses per minute. The rats were then trained to maintain stable rates, defined as 10 to 20% of the baseline rate, followed by a gradual decay to a much reduced rate, or zero. In some cases this was followed by full or partial resumption. Resumption rates of 180 μA, however, were not likely to be followed by a return to the lever. During the period when the effect was seen, the animals were tested neither reactively 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 animal no longer attended to the lever. As Loriesal 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 cells.

AFFERENTS TO AREA 8 AND OTHER PREFRONTAL AREAS IN THE MONKEY AND BABOON. An HRF STUDY. George R. Lelchnetz 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 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 afferent systems in primates, and this report represents preliminary findings from 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 (3-5 ul, 50% sol. in sterile saline. Sigma Type V) 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 isotonic saline followed by a mixed aldehyde fixative (10% formaldehyde, 1.25% glutaraldehyde) in a 0.1M phosphate buffer. The brains were removed, blocked, and immersed in a 10% sucrose/buffer solution overnight and then cryostat-sliced at 40 μm, and were reacted according to the Mesulam (1976) blue-reaction protocol. The mesulam included the following steps: 1) incubation at room temperature for 6 hours in a 1 or 5% solution of 3H-HRP or 2) incubation at 4°C for 6 hours in a 1% solution of 3H-HRP. The brains were then sectioned 40 μm and were processed for autoradiograms under darkfield light microscopy.

LONG-TERM LIMBIC STIMULATION WITHOUT KINDLING OF SEIZURES. Henry Leese 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 automatons 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 afterdischarges, interictal spikes). Detection thresholds (the minimal current required to elicit an appropriate 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 structures or to other abnormalities were observed. 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 seizures and kindling changes in the midline and other abnormalities can 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.)
EVOKE POTENTIALS IN HIPPOCAMPAL CA1 IN ANESTHETIZED AND WAKING RATS. Li Shen Leng. Dept. Physiol.-Anat., UC Berkeley, CA 94720.

Three inputs -- the posterior alveus (PA), the anterior alveus (AA) and the so-called collector alveus (SCA) -- were studied in rats anesthetized with sodium pentobarbital, using near-threshold electrical stimuli. Averaged evoked potentials (AEPs) were mapped in the pyramidal and interneuronal and areas generated by inhibitory post synaptic potentials (IPSPs) in pyramidal (PYR) cells could be inferred for each stimulus. The sink for the IPSP field could be evoked by the alveus stimulus (AA,PA) was at stratum oriens and a positivity 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 stimulus evoked a broad surface distribution of AEP which suggested significant spatial divergence of the orthodromic inputs in contrast to the sharp projection of the causally directed efferent fibers in PA.

In implanted, awake animals, AEPS showed significant changes. For AA and PA stimulation, the late component inferred as the IPSP field become smaller while the early EPSP field was enhanced. For Sch stimulation, 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 NSF GB 35532 and NIH IF 32 NS05874-01.)

AFFERENTS TO THE INTERPEDUNCULAR NUCLEUS OF THE RAT. L. Marchand, J. F. DeFrance, and J. C. Stanley. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, P.O. Box 20700, Houston, Texas 77025.

Field and extracellular potentials were recorded in the ventromedial nucleus of the hypothalamus (VMH) after stimulation in the fimbria and stria terminalis at the level of the dorsal hippocampus. Recorded and stimulating electrodes were glass micro electrodes filled with 2M NaCl and fast green for later localization of tip position (Thomasson and Wilson, 1965). Animals were anesthetized with urethane (100 mg, i.v.). 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 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 (25-50um) 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.

Afferents from the habenular nuclei appear to arise exclusively from the medial habenular nucleus, raphe nuclei (nucleus central superior and nucleus dorsalis), dorsal and probably ventral tegmental nucleus, parabigeminal nucleus and the locus coerulent. 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 TO THE INTERPEDUNCULAR NUCLEUS OF THE RAT. R. Marchand, J. R. Riley, and R. Y. Moore. Dept. of Neurosciences, School of Medicine, Univ. Calif., San Diego, La Jolla, CA 92037.

The interpeduncular nucleus (IPN) receives its major input from the habenular nuclei. Recent studies indicate that fibers of other afferents may undergo sprouting. The IPN, follow ing lesioning of the habenular nuclei, It seemed worthwhile, therefore, to further characterize the afferent connections of the IPN in order to define the candidate structures for this observed morphological and biochemical plasticity.

Heresroidial 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.

Activity product was observed in neurons of the medial habenular nucleus, raphe nuclei (nucleus central superior and nucleus dorsalis), dorsal and probably ventral tegmental nucleus, parabigeminal nucleus and the locus coerulent. 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.

Reaction product was observed in neurons of the medial habenular nucleus, raphe nuclei (nucleus central superior and nucleus dorsalis), dorsal and probably ventral tegmental nucleus, parabigeminal nucleus and the locus coerulent. 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.

Amygdaloid Modulation of Wheel Running Acquisition. Irwin N. Lourie*, Michael M. Krieger* (SPON: N. S. Thampi). Research Laboratory, Horsham State Hospital, Horsham, PA 19040.

Using females from an inbred strain of Sprague-Dawley derived rats selectively bred for high activity wheel levels for 18 generations, the ability of a new paradigm was tested. 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 radiofrecency technique in 4 subjects. An equal number of sham operated controls (C) were produced for a group of rats. After a period of recovery for recovery and partial extinction of the activity pattern, reacquisition of wheel running was measured over a period of 14 days. In addition to measurement of circadian rhythms, 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 occurs and 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 inhibition 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 concomitant deprevation state due to food or water restriction. The IPN, following lesioning of the habenular nuclei. It seemed worthwhile, therefore, to further characterize the afferent connections of the IPN in order to define the candidate structures for this observed morphological and biochemical plasticity.

Heresroidial 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.

Activity product was observed in neurons of the medial habenular nucleus, raphe nuclei (nucleus central superior and nucleus dorsalis), dorsal and probably ventral tegmental nucleus, parabigeminal nucleus and the locus coerulent. 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.

Afferrents to the habenular nuclei appear to arise exclusively from the medial cell group. Following injections restricted to the IPN, only a small number of medial habenular nuclei was 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 of the brainstem. The labeled neurons appear 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 rostral dorsal are labelled 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 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 from the medial habenular nucleus and restricted portions of the dorsal tegmental nucleus.
Dissociations between hypothalamic knife cuts affecting sexual behavior, pup retrieval, and object retrieval in female hamsters. David H. Nusbaum, Charles M. Marks, and Juanna Thomas.

This study explores the roles of the lateral connections of the medial hypothalamus in hamster pup retrieval, pup cannibalism, nest building, and object retrieval. In this study, female hamsters were pretreated for their response to pups as virgins, then received sagittal cuts either lateral to the anterior hypothalamic area (anterior cuts) or lateral to the median anterior hypothalamic-ventromedial nucleus (posterior cuts). Postoperatively, females were tested a) as virgins, for changes in pup retrieval and cannibalism, b) as mating behavior, c) for maternal behavior, and d) for object retrieval.

Cross correlation analysis is carried out on electrical activity in the sigmoid gyrus, nucleus accumbens and amygdala. The studies are carried out on rats with dorsal raphe lesions or fornix lesions. Cross correlation analysis shows the direction of signal flow (40 Hz) between the sigmoid gyrus and nucleus accumbens and amygdala.

In order to investigate the function of indolealkylamines, in particular N-Acetylseryotonin (NAS), a sensitive immunohistochemical method has been developed and antibodies against NAS and Melatonin have been raised. NAS in the hippocampus, in particular N-Acetylserotonin (NAS), in brain, we have developed a specific anti-NAS antibody to extend an earlier observation which suggested the presence of N-Acetylindolealkylamines (NAI's) in the hippocampus.

The antibody directed against melatonin, however, did not stain the hippocampus in any detectable fashion, leading to the conclusion that the visualized NAS and melatonin was produced in rabbits using NAS coupled to bovine serum albumin (BSA) via the Mannich reaction, (Br.Res.81, 196, 1974). Antiserum to NAS was produced using, as antigen, NAS coupled to BSA via a pararcarboxbenzylyl bridge attached at the indole nitrogen (unpubl.). The first two antibodies were 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.

Several 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. A normal rabbit serum contained against 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, however, an antigen, NAS coupled to BSA via a pararcarboxbenzylyl 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.

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 occurrence of behaviors such as walking and rearing (Type I behaviors). In addition, confusion of the two different mechanisms, i.e., the possible involvement of 6-OHDA in the production of RSA and LVF, remains to be investigated.

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 occurrence of behaviors such as walking and rearing (Type I behaviors). In addition, confusion of the two different mechanisms, i.e., the possible involvement of 6-OHDA in the production of RSA and LVF, remains to be investigated.
710 FURTHER OBSERVATIONS ON GRANULE CELL DEGENERATION FOLLOWING ANODAL DIRECT CURRENT LESIONS. Russell E. Ruth, Scott Cain, Anthony DiGianfilippo and Arvy Routtenberg. (Supported by MH 25281 and NSF 19388 to A. R.)

We recently described extensive granule cell degeneration (GCD) following systemic, 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) GCD lesions localized the spread of the lesion roughly to a lamellar plane of Blackstad et al. (J. comp. Neurol., 130, 1970, 433-450); (ii) this reaction was detectable light-microscopically between 3 - 3 hrs post lesion; (iii) neither radio-frequency (heat) nor microknife lesions provoked GCD outside the lesion boundary. Several additional observations have been made. 5 µA a-DC is not sufficient to produce GCD. The severity of GCD appears greater in younger animals (25 days old). Spontaneous discharge rates of granule cells following a-DC lesions were recorded 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, 227-228). (Supported by NIMH grant MH19691).


Intraventricular kainic acid (KA) destroys rat hippocampal pyramidal cells with great potency. CA3 pyramidal cells are most sensitive; KA penetrates 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 property (Ryan et al., 1979), we have investigated beyond this the primary lesion. (for 2 min) extensive GCD can be observed. The severity of GCD appears greater in younger animals (25 days old). Spontaneous discharge rates of granule cells following a-DC lesions were recorded 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, 227-228). (Supported by NIMH grant MH19691).


Systemic administration of KA has effects on widespread regions of the brain, the extent of the lesion can vary even among animals that received the same dose of KA. Kainic acid blocks the action of glutamate and its analogs (Olney et al., 1979). We have surveyed the effects of systemic or intracerebral injections of KA on the forebrain of rats using frozen sections stained with the MAb method or the cupric-silver method of De Olmos to reveal areas of cellular damage and agrophyllosis. Systemic administration of KA has effects on widespread regions of the forebrain although the extent of the lesion can vary even among animals that received the same dose of KA. Agrophyllous neurons and/or cellular debris and gliosis are seen especially in parts of the hippocampal formation, septum, olfactory cortex, amygdaloïd complex, middorsal and mediolateral 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 widely 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 injection of KA. However, intracerebral injections of KA into the posterior ploriform cortex (PC) or entorhinal area (EA) affect CA1 more severely than the excitatory actions related 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 the hippocampus also destroy the corresponding thalamic nucleus (e.g. the ectorhyme and ventral 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 limits of PC (apatracta), in some cases contrastralateral to the injection. These results are difficult to explain on the basis of diffusion and/or differential sensitivity and suggest that the patterns of damage are related to axonal connections between the affected areas. (Supported by NIMH grants NS05918, GM07200, ES07066).

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, and post-tetanic potentiation (PTP) 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 kOhm) 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 (6 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 2 Hz and reached a plateau at 8-12 Hz. Both slow-wave responses to cCA3 and MSR which decrease more slowly thereafter, baselines were generally reached after 8-10 seconds. The population spike latency of TP which decays rapidly in the first two seconds following the tetanus and decayed more slowly thereafter, baseline responses were generally reached after 8-10 seconds. The population spike latency of TP lasting several seconds. cCA3 test responses were enhanced 10-20% following the tetanus train were recorded for study. TP was observed in the 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 to cCA3 and MSR do not last passing 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 R05 38532 to JDF.

VALIUM: A SUPPRESSOR OF HIPPOCAMPAL PYRAMIDAL CELL EXCITABILITY. R. 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 20706, 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 cell excitability, and may do so by suppressing cyclic GMP mechanisms.

Supported by ISF GB35532 and Hoffman-LaRoche, Nutley, N.J.


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 cyologically distinct subgroups. A total of eight paired and unpaired subgroups are identifiable. The autoradiographic tracing method reveals that these subgroups 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 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. Autoradiography, micropuncture injections with biocytin and HRP methods were used to determine whether these various efferent connections arise from specific IPC subgroups. Tracer labeled leucine and biocytin 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 specific efferent projections within the total pattern of IPC efferent projections.

These results reveal IPC efferent projections that are new or different from previous reports. Major 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.


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 lesions were found to project 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 NS 25495.

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 projections between the subicular area of the hippocampal formation and several cortical areas of the temporal lobe. Although these studies have established that hippocampal projections can be visualized in the primate, much more needs to be learned about this link. In this study cortical projections to the entorhinal cortex were investigated in the rhesus monkey using an autoradiographic method. All major architectonic subdivisions of the temporal lobe were sampled with the exception of the primary auditory cortex.

Four areas have been identified which send direct, powerful projections to the entorhinal area. Individually, these correspond to Brodmann's areas 35 and TH and TG. Projections from areas TG and TH are directed primarily to the lateral entorhinal area and a sizable zone previously called the intermediate entorhinal area. Projections from areas 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-3. In addition to these projections, areas TP, 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 the most 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 that 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.

DIFFERENTIAL EFFECTS OF LIMBIC SYSTEM LESIONS ON LONG-TERM AND SHORT-TERM SPATIAL MEMORY IN THE RAT. John A. Walker (spn: 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 flexible use of memory. The present experiment was conducted to determine if the memory requirements of spatial tasks influence the retention of those tasks following limbic system damage. Two tasks were developed, requiring either memory for only one goal within a test (a short-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 short-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 goal was the rewarded goal. Care was taken to prevent simple position habits from providing a solution to the tasks. Rats in both groups continued to perform accurately after surgery, with the exception of few enzyme-reactive processes running across it. Radiotracers and autoradiographs were used to label the hippocampal connections to cortical structures, or sham operations. Testing was resumed after a week of recovery.

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. The region of the mammillary 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 with those obtained in the anesthetized preparations.

Supported by NIH Grant NS 07941-09.
ULTRASTRUCTURAL EVIDENCE FOR CHANGES IN THE X-IRRADIATED RAT HIPPOCAMPUS


Previous studies (Wallace, Kaplan & Werboff, 1976, Exp Brain Res.; Neurosci. Abstr., 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 Tetroxide, stained en block in uranyl acetate, and embedded in Epon-Araldite. Toluidine blue 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 removed and the dentate gyrus and area CA3 dissected 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.

AUTORADIOGRAPHIC MAPPING OF BRAIN REGIONS ACTIVATED DURING SELF-STIMULATION USING 14C-2-DEOXY-D-GLUCOSE.


Seven self-stimulating rats were injected via intracardiac catheters with 14C-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 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 the 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 USPHS Grant NS 05863 and by a grant from the Sloan Foundation.
MEMBRANE BIOPHYSICS
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 stimuli, 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 frequencies (e.g. 1 Hz) remains constant. This means that, at a given voltage, the number of open channels is competition between the activation and inactivation rate constants of two exponentials having time constants of about 0.5 sec. and 2 sec. respectively. To account for the apparent cumulative inactivation during the inter-pulse interval, MacKinnon and Lux (Science, 197, 472-475, 1977) postulated that cumulative inactivation is an independent component of outward current termed K-current and that accumulation of calcium ions is a phenomenon cumulative inactivation. To account for the apparent cumulative inactivation, we have found 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 sec.

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 activation during the inter-pulse 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.

Further, they point towards an important contribution of the Na+-Ca++ exchange pump in the removal of free cellular Ca++ ions. The inhibition of Na+-Ca++ exchange should increase the rate of removal of free cellular 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 Ca++ current followed a time course similar to changes in arsenazo III 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, Proc. 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 -40 mV from a step to 0 mV. 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 summation exponentials (TCEs) in normal ASW of 0.74 and 4.18 seconds. In Tris (0 Na+) ASW, the faster component remained essentially unaltered (TC; 0.74 sec.) while the slower time constant was increased (TC; 5.71 sec.).

The inhibition of Na+-Ca++ exchange should increase the concentration of Ca++ in the membrane and cause this experiment suggests that 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 to the role of the Na+-Ca++ exchange pump in the removal of free Ca++ following Ca++ influx.

MEMBRANE BIOPHYSICS


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. Extraction of calcium-45 from muscle and ganglion cells. This finding suggests that a slow component of Ca++ current was studied both in the presence of Mn++ and in the presence of Mn++-Ca++ exchange pump (Baker, 1972, Proc. 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 -40 mV from a step to 0 mV. 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 summation exponentials (TCEs) in normal ASW of 0.74 and 4.18 seconds. In Tris (0 Na+) ASW, the faster component remained essentially unaltered (TC; 0.74 sec.) while the slower time constant was increased (TC; 5.71 sec.).

The inhibition of Na+-Ca++ exchange should increase the concentration of Ca++ in the membrane and cause this experiment suggests that 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 to the role of the Na+-Ca++ exchange pump in the removal of free Ca++ following Ca++ influx.

Diary...
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 subsequent disengagement 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 the fission occurs. Such a rise in local tension would be transient because lateral diffusion of lipid molecules from adjacent regions would relieve the tension. This simple model between the two hypothetical 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 the already existing area, otherwise the tension cannot reach threshold and fission cannot occur.

2. The entry of Ca will cause vesicles to the terminal membrane and to each other which may aid in the flattening of those vesicles opening to the synaptic cleft. As indicated above, lateral lipid diffusion may then result in fusion between local vesicle in the flattened region. Since there is strong evidence that i.0 Hz stimulation, there are no vesicle loss but rather that vesicles are immediately reutilized (e.g. to release newly synthesized acetylcholine).

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.

MODELLING THE EFFECTS OF TONIC CONDUCTANCE CHANGES ON EPSP's AND SO. A STRANGULAR CURRENT PULSE - WAYS TO ESTIMATE THE LOCATION AND EXTENT OF NEURONAL CONDUCTANCE CHANGES DUE TO LO. LASTING POSTSYNAPTIC POTENTIALS OR DRUGS. Peter L. Carlen and William A. Corrigall, Neurobiology Lab. Addiction Research Foundation, Toronto, Canada, and University of Toronto.

Tonic conductance (G) changes which result from temporally sustained or long-lasting depolarizations play an important role in central neuronal integration. Using the short intracellular somatic 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 G dendrites. 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 Hall, B. (Ph.D. thesis, 1975, Dept. Electrical Engineering, Monash University, Australia) with a time constant of 12 msec, and an electronic length of 1.5 λ (time constant) was used.

CA-DEPENDENT INACTIVATION OF CALCIUM CONDUCTANCE IN PARAMECIUM. Paul Breger* and Roger Roberg (SPON: Douglas Junge). Biology Dept. UCLA, Los Angeles, CA 90024.

Inactivation of early inward current (ICa) was examined in voltage-clamped C. caudatum. In 1.0 mM Ca, depolarizations of 5 to 25 mV from a holding potential of -75 mV resulted in 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 conductance.

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 interpulse interval (Fig. 1). 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 ICa during PII. A further increase of PI amplitude toward the calculated ECa (Ca = 150 mV) resulted in a return of the early ICa during PII to 90% of its control value. This increase in ICa during PII prevented PI amplitude indicating no substantial persistent-Ca activated I Ca. Substitution of Ca by Na decreased inactivation of the early inward current. In 0.1 mM Na, 50 ms pulses of PI (inactivation see Fig.). Injection of EHTA slows inactivation of ICa and also increases peak ICa.

During a 100 ms, 20 mV depolarization the inward current shows substantial time-dependent inactivation in a 5 Na, 0.1 Ca solution. This inactivation may result from either Na influx, Ca influx, or direct voltage effects on the channel. A 120 mV, 100 ms pre-pulse, 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.
VOLTAGE CONTROLLED GAP JUNCTIONS BETWEEN EMBRYONIC CELLS: A VOL-
TAGE CLAMP STUDY. A.L. Harris*, D.C. Spray, N.V.L. Bennett, & R.B. Han
tural Studies, SUNY, Syracuse, N.Y.
Current clamp studies reveal voltage dependence of electrical coupling
between blastomeres of early amphibian (Apterygium) blastula. 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 junc-
tional 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 across the cells. Typically, junctional conductance (1-4 µmho) drops to less than 10% of its resting level with a 20 mV 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 re-
sistance. 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 the junctional voltage is stepped to voltages of opposite sign, each of which is sufficient to cause low conductance. We believe that the conductance elements which underlie this pheno-
menon are not homogeneously distributed across the gap junctional membranes. The decrease of the HAP in NE occurred

ACTION OF 4-AMINOPIRYLIDINE ON VOLTAGE-AND CALCIUM-DEPENDENT
POTASSIUM CURRENTS OF MOLLUSCAN PACEMAKER NEURONS. Anton
Hermans* and A.L.F. Gorman, Dept. of Physiology, Boston U. Sch. of
Med., Boston, MA 02118.
Neuronal soma membranes exhibit both, a voltage-dependent pot-
asium-current, \( I_{k}(v) \) and a calcium-dependent potassium-current, \( I_{k(Ca)} \). These current components in Aplysia pacemaker neurons are separated as indicated previously (Hermans 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 \( I_{k(Ca)} \) (activated by voltage steps of 200 msec dura-
tion in zero Ca\(^{2+}\) solution) in a dosage dependent manner. The
blockage was voltage dependent. No blockage of \( I_{k}(v) \) was ob-
erved by intracellular iontophoretic injection of Ca\(^{2+}\), how-
ever was observed; rather with higher concentrations of 4-AP this current component was increased. These results are consis-
tent 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 a dosage dependent manner (EC\(_{50} = 1-2 \mu M\)). We concluded that NE inhibits

NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT
SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of
Physiol. & Biophys. U. of Miami Sohl. of Med., Miami, FL 33152, and Div. of
Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.
Partial blockage of the rat sympathetic postganglionic neuron results in a hyperpolarising afterpotential (HAP). Wawrosky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused by an increased g\(^{Ca}\) triggered by Ca\(^{2+}\) influx during the depolarization. We now report that norepine-
phrine inhibits this process.

INTRACELLULAR RECORDING FROM THE BRAINS OF CONSCIOUS BEHAVING
CATS ACHIEVED BY A NEW METHOD OF FLOATING THE PIPE TIP. Gregory L.
ol. Baylor College of Medicine, Houston, Texas 77030, U.S.A.
We have been working on developing a method to record intracellularly from the brains of conscious behaving cats. The problem is to minimize the cardiovascular- and respiratory-induced movements that occur during recordings. A new method using a closed-cranium method utilizing agar and solved the limb

NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT
SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of
Physiol. & Biophys. U. of Miami Sohl. of Med., Miami, FL 33152, and Div. of
Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.
Partial blockage of the rat sympathetic postganglionic neuron results in a hyperpolarising afterpotential (HAP). Wawrosky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused by an increased g\(^{Ca}\) triggered by Ca\(^{2+}\) influx during the depolarization. We now report that norepine-
phrine inhibits this process.

INTRACELLULAR RECORDING FROM THE BRAINS OF CONSCIOUS BEHAVING
CATS ACHIEVED BY A NEW METHOD OF FLOATING THE PIPE TIP. Gregory L.
ol. Baylor College of Medicine, Houston, Texas 77030, U.S.A.
We have been working on developing a method to record intracellularly from the brains of conscious behaving cats. The problem is to minimize the cardiovascular- and respiratory-induced movements that occur during recordings. A new method using a closed-cranium method utilizing agar and solved the limb

NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT
SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of
Physiol. & Biophys. U. of Miami Sohl. of Med., Miami, FL 33152, and Div. of
Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.
Partial blockage of the rat sympathetic postganglionic neuron results in a hyperpolarising afterpotential (HAP). Wawrosky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused by an increased g\(^{Ca}\) triggered by Ca\(^{2+}\) influx during the depolarization. We now report that norepine-
phrine inhibits this process.

INTRACELLULAR RECORDING FROM THE BRAINS OF CONSCIOUS BEHAVING
CATS ACHIEVED BY A NEW METHOD OF FLOATING THE PIPE TIP. Gregory L.
ol. Baylor College of Medicine, Houston, Texas 77030, U.S.A.
We have been working on developing a method to record intracellularly from the brains of conscious behaving cats. The problem is to minimize the cardiovascular- and respiratory-induced movements that occur during recordings. A new method using a closed-cranium method utilizing agar and solved the limb

NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT
SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of
Physiol. & Biophys. U. of Miami Sohl. of Med., Miami, FL 33152, and Div. of
Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.
Partial blockage of the rat sympathetic postganglionic neuron results in a hyperpolarising afterpotential (HAP). Wawrosky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused by an increased g\(^{Ca}\) triggered by Ca\(^{2+}\) influx during the depolarization. We now report that norepine-
phrine inhibits this process.

INTRACELLULAR RECORDING FROM THE BRAINS OF CONSCIOUS BEHAVING
CATS ACHIEVED BY A NEW METHOD OF FLOATING THE PIPE TIP. Gregory L.
ol. Baylor College of Medicine, Houston, Texas 77030, U.S.A.
We have been working on developing a method to record intracellularly from the brains of conscious behaving cats. The problem is to minimize the cardiovascular- and respiratory-induced movements that occur during recordings. A new method using a closed-cranium method utilizing agar and solved the limb

NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT
SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of
Physiol. & Biophys. U. of Miami Sohl. of Med., Miami, FL 33152, and Div. of
Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.
Partial blockage of the rat sympathetic postganglionic neuron results in a hyperpolarising afterpotential (HAP). Wawrosky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused by an increased g\(^{Ca}\) triggered by Ca\(^{2+}\) influx during the depolarization. We now report that norepine-
phrine inhibits this process.

INTRACELLULAR RECORDING FROM THE BRAINS OF CONSCIOUS BEHAVING
CATS ACHIEVED BY A NEW METHOD OF FLOATING THE PIPE TIP. Gregory L.
ol. Baylor College of Medicine, Houston, Texas 77030, U.S.A.
We have been working on developing a method to record intracellularly from the brains of conscious behaving cats. The problem is to minimize the cardiovascular- and respiratory-induced movements that occur during recordings. A new method using a closed-cranium method utilizing agar and solved the limb

NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT
SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of
Physiol. & Biophys. U. of Miami Sohl. of Med., Miami, FL 33152, and Div. of
Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.
Partial blockage of the rat sympathetic postganglionic neuron results in a hyperpolarising afterpotential (HAP). Wawrosky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused by an increased g\(^{Ca}\) triggered by Ca\(^{2+}\) influx during the depolarization. We now report that norepine-
phrine inhibits this process.

Previous work from this laboratory showed that tetrodotoxin (TTX) prolonged the action potentials of Retzius and N, P and T sensory leech neurons by blocking a K current which normally repolarizes them. As a consequence, a late divergence of the action potential was observed which dominated the late behavior during excitation. The order of responsiveness of the neurons to TTX was 

M > N > P > T which paralleled their ability to sustain active membrane responses in high potassium 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).

In the present work we undertook 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; Schaaf, 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 K 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 Na. These findings suggest that a late portion of the action potential of these leech neurons was blocked by SPT which normally generates only small graded responses when depolarized. 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 results indicated that the stimulation of active membrane responses in Na-free SPT solutions were dependent on extracellular Ca. Sr was capable of replacing Ca as a current carrier, while Mn, known to block divalent cation responses in leech R and H cells, abolished them. Intracellular injection of SPT by pressure had essentially no effect in K, P and T cells, in contrast to intracellular TTX 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.

The pharmacological block of K currents provide a useful method for the study of Ca channels in individual neurons and further our understanding of their responses to drugs.


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. 95, 1975). The possibility that a change in intracellular pH might in part mediate the effects of alterations in cell energy metabolism on membrane calcium electrogenesis has been investigated. Intracellular pH was measured by means of microelectrodes (Thomas, J. Physiol. 1974; 238:159). Lowering the extracellular pH from 7.4 to 5.6 had little effect on internal pH or fiber electrical properties. Exposure to 100% CO2-CO2 HCO3 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 CO2 exposure, the small graded electrical responses of the fibers were converted into overshooting action potentials. Effects of CO2 on internal pH and electrical properties were readily reversible upon perfusion with 100% O2-0% CO2 (J. Physiol.). Linearization of steady-state constant-current I-V plots obtained in Ca++-free, Mn++ Ringer's upon CO2 exposure suggests that the decrease in internal pH blocks delayed rectification. This finding may indicate a blockage of a voltage-dependent potassium current which normally shunts the inward Ca++ current and limits the size of the depolarizing response in the fibers. Effects of internal pH on the inward current were not studied. Another, possible means of producing internal acidification while the fiber is exposed to the normal extracellular solution is by brief exposure to pH < 7.4 after brief exposure. The internal pH of the slow flexor fibers increased 0.5-0.8 units upon exposure to 30-50mM NH4Cl at pH 7.4. Removal of NH4Cl after 3-5min exposure produced little change in internal pH below the pre-NH4 value by 0.5-1.0 units. Preliminary results indicate that this NH4+ rebound acidification develops, a gradual repolarization of internal pH changes occurs, with all-or-none spikes appearing at internal pH values below about 6.3. Evidence relating the electrophysiologic changes during NH4+ rebound to the role of internal pH changes in other cells, will be discussed. Supported by USPHS 09012 to S. Nagiwa and a Helen Hay Whitney Foundation postdoctoral fellowship to the author.


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 (10-9M), the resting potential underlying cycles of spontaneous depolarization was followed by repolarization, producing an undulating pattern. Repetitive firing occurred both spontaneously and in response to a single stimulus. The rate of rise amplitude during a depolarizing phase of the action potential were unaffected but the late falling phases were replaced by a long plateau. At higher concentrations of AP-A (10-7 - 10-9M), 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 + 300mM TEA; 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 A. sulcata (ATA II), prior treatment with TX and procaine did not affect subsequent interaction of AP-A with its receptor. As the TX effect was reversed on washing, the action potential returned and had 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 10-4-10-7mM TTX. After washout of TTX, depolarization appeared normal but inactivation was markedly slowed in the presence of 10-7M AP-A. The peak current was slightly reduced at lower AP-A application. Concentration potentiation of this inactivation by external application provides another useful tool in the pharmacologic dissection of ionic channels. The close structural similarity between AP-A and ATA II suggests that AP-A might activate the same sodium channels of membrane currents and action potentials are contrasted by their quite different interactions with TTX. This difference, when elucidated, may provide insights into the complex relationship of TTX receptor and the sodium channel. Supported by NIH grant 54144.


In order to better define the physical properties of the extracellular space of the brain, and provide a reference for the movement of macromolecules in vivo, the apparent diffusion coefficient, D*, for water, Na+, K+, and some other ions were determined both intracellularly and extracellularly. Using ion-selective micropipettes containing Corning 477317 ion exchange micropipettes of MgCl2 as a function of depth was highly consistent with Pick's law of diffusion in a semi-infinite, homogeneous medium. In some cases, however, the profile was more consistent with Fickian diffusion in a semi-infinite, homogeneous medium. In contrast, D* for K+ in the cerebellar cortex was only 2-3 times the value of D* in water. In contrast, D* for K+ in the cerebellar cortex was only 2-3 times the value of D* in water. This difference, when elucidated, may provide insights into the complex relationship of TTX receptor and the sodium channel. Supported by NIH grant 54144.

* Supported by Public Health Service, Grant NS-13742.
VOLTAGE-SENSITIVE Ca-CHANNELS AND Ba ION IN PARAMECIUM TETRAURELIA. Youko Satow. Lab. Molecular Biology, Univ. of Wisconsin, Madison, WI 53706.

Transient inward current (Iin) in Paramecium tetraurelia recorded upon a step depolarization with a voltage clamp is normally carried by Ca++. The maximal inward transient (Imax) 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++]0 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 Iin appears to be insensitive to further addition of Ba in terms of Imax. The 1.5 mM Ca-solution (or over 1.5 mM Ca) gives the highest Imax in Paramecium. The Imax is smaller in a Ca-solution with 0.75 mM Ca-2 mM Ba and 3.5 mM Ca-2 mM Ba solutions, respectively.

Ionophoretically injected Ba++ reduces the Imax. 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 suggest that Ba++ rather than inhibits 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 C. Kung.


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 occurs at periphasic potentials and is carried by 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 2,6-dimethylphenol, 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 tetrodactylylamin (TTX), 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 Iin and steady K+ currents. (Supported by V.A. research grant NRR1)[160.]

EFFECTS OF EXTERNAL calcium AND calcium BLOCKING AGENTS ON THE PHOTORESPONSE IN LIMULUS VISUAL PROTOPHYTOPOCEREBRIT. Jeffrey A. Schmidt* and Alan Field† (SPOM: C. Kung). Lab. of Physiological Chemistry, IRP, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

The effects of Ca++ on the voltage sensitivity of extracellular calcium, [Ca++]o, on the light-evoked discrete wave responses (bumps) were measured. Ca-blocking agents CdCl2 (2 mM) or D-600 (10-3M) raised the membrane potential and increased the depolarizing amplitude and response latency, while reduction in [Ca++]o reversibly increased both parameters. A slowing of the bump repolarization occurred with these blocking agents but was not reversed [Ca++]o hyperpolarisation which followed the de­

polarizing transient becomes larger under steady depolarisation. The amplitude of the hyperpolarization was correlated with ampli­
tude of the depolarising transient on top of steady depolarisation of +10mV, and was eliminated when the membrane was hyperpolarised. The hyperpolarisation was smaller in low [Ca++]o when normalized to the depolarisation amplitude, and was eliminated by the Ca-blocking
tagents and by the K-blocking agent quinidine (10-4M).

The magnitude of outward tail currents measured under voltage clamp following depolarisation depend on duration and magnitude of the depolarisation. The outward currents appeared maximal at de­
polarising pulse durations approximating bump durations. D-600 and quinidine reversibly suppressed the tail currents as well as the outward current during the depolarisation. Reversal of the tail current occurred at a potential negative relative to resting poten­
tial.

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 hyperpolarisation results from a Ca-dependent rise in K conductance that may serve to actively repolarize the photoreceptor in the unclamped receptor.

Superfusion of IBMX (5mM), a phosphodiesterase inhibitor, quality­

mimicked the effect of these two blocking agents on both the currents and the depolarisation. Hyperpolarisation and cyclic nucleotide metabolism may play a role in generation of the Ca-dependent outward current.

The amplitude of light-evoked bumps and photoreponses elicited by dim flashes could be mimicked by intracellular current injec­
tion. 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 de­
polarisation and is not produced in direct response to light. (Partially supported by a Grass Fellowship given to J.A.B.)

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 the axons of the annelid P. anguilla. In TEA-substituted zero-sodium medium, we record a sustained inward current which does not facilitate to successive 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 depolarization. The frequency of response maximum (usually about 150 Hz) was extending usually from 100 to 200 Hz at room temperature (19ºC). The frequency spectra of these responses were always broad, and the number of membrane sites involved in production of miniature responses. With scorpion venoms, etc. were employed to evoke these "periodic miniatures responses." The frequency of periodic responses at different membrane sites. As 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 mV, 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 potentials was 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 voltage-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.

VOLTAGE CONTROLLED RESISTANCE AND PERMEABILITY OF GAP JUNCTIONS BETWEEN EMBRYONIC CELLS. D.C. Spray, A.L. Harris*, N.V.L. Bennett, P.C. Hulme, Dept. Neurosci., A. Einstein Coll. Med., Bronx, N.Y. Blastomeres (32-cell stage to morula) of the axolotl Ambystoma maxicanum 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 e- 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 e-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 calcium conductance and more moderate sized molecules. Long hyperpolarizing current pulses through an electrode containing Lucifer Yellow CH (MW 443) simultaneously held pairs of iso-coupled and iso-eccentric 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 coupling potential in different regions an embryo could rapidly interrupt communication to allow independent development. DCS is a McKnight Scholar.


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 the axons of the annelid P. anguilla. In TEA-substituted zero-sodium medium, we record a sustained inward current which does not facilitate to successive 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 depolarization. The frequency of response maximum (usually about 150 Hz) was extending usually from 100 to 200 Hz at room temperature (19ºC). The frequency spectra of these responses were always broad, and the number of membrane sites involved in production of miniature responses. With scorpion venoms, etc. were employed to evoke these "periodic minatures responses." The frequency of periodic responses at different membrane sites. As 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 mV, 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 potentials was 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 voltage-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.

A CAUTION REGARDING THE USE OF TEA AND COBALT TO SEPARATE VOLTAGE-DEPENDENT AND CA-DEPENDENT OUTWARD CURRENTS. S. R. 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 giant axons 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 mV TEA blocks 95% 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. C-current can then be isolated by a single exponential, which 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.
Inactivation of calcium current \( I_{\text{Ca}} \) is revealed when interference by other ionic currents is eliminated. \( I_{\text{Ca}} \) 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 syntanin. This eliminates effects due to changes in both the voltage- and calcium-activated Ƙ channels. Further, all test biotic nystatin. This eliminates effects due to changes in both

**+**

These data indicate that it is the calcium entry associated with 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 +200 mV. For inactivation of \( I_{\text{Ca}} \), PI is increased with PI II voltage increases toward putative ECa (ca +140mV). When barium solutions contained no Na or K. The Figure represents show representative data from a double-pulse experiment on R-15 recorded with a hold potential of +400mV 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 +200 mV. For inactivation of \( I_{\text{Ca}} \), PI is increased with PI II voltage increases toward putative ECa (ca +140mV). When barium was substituted for bathing Cs 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 \( I_{\text{Ca}} \) during PII. NIH 5NS363 and DOTTN70209.

**CaO Na**

### PENICILLIN BLOCKS INHIBITORY CONTROL OF DENDRITIC BURST GENERATION IN HIPPOCAMPAL NEURONS


Previous studies have shown that epileptiform field potentials occur spontaneously in CA1 and CA3 regions of hippocampal slices exposed to penicillin in vitro. Intracellular recordings show that neurons in these areas generate depolarization shifts (DS) and spike bursts coincident with spontaneous or orthodromically evoked epileptic field potentials. Data suggest that DS are intrinsically generated in dendrites. In contrast, CA1 neurons from slices bathed in normal medium generate neither spontaneous nor orthodromic evoked bursts. In order to determine how penicillin alters the behavior of CA1 cells recording 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 small EPSPs. 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 a decrease in dendritic burst generation by interfering with the inhibitory mechanism on dendrites which normally serves to block the occurrence of dendritic burst generation during orthodromic stimulation of these neurons. (Supported by NIH Grant NS06477.)

**100 CaO Na**

### TEMPERATURE ACCLIMATION ALTERS MEMBRANE PROPERTIES OF IDENTIFIED NEURON IN THE LIME SNAIL, HELOIX PHELACONICOLA AND HERBIVOROUS L. 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 heliox neurons are active throughout the year, a series of characteristic changes in electrical and functional properties occurs with acclimation to different temperature ranges. These changes are not simply adaptable to cooler temperatures, but are also attributable to the effects of temperature acclimation on several properties of a neuron from E. malleus. The large, easily identifiable bullying neuron in the isolated right parietal ganglion of snails acclimated to 5°C generally caused: 1) cessation of spontaneous activity, 2) decrease in excitability, 3) increase in Rm, 4) prolongation and slight increase in amplitude of individual action potentials, 5) decrease in calculated electrogenic pump current, 6) decrease in absolute passive Na-permeability, 7) change in cation permselectivity such that PN a /Pk decreased 75%, but PC s / P K and PRb /PK 50% and 15% less than in cells acclimated to 20°C. In sum, the thermal acclimation of snails is reflected in membrane biophysical properties such as permselectivity to different ions.

**100 CaO Na**


The use of single, isolated cells makes it possible to study the electrical properties of smooth muscle without such complications as low resistance, extracellular contamination of neural elements found in tissue. Tissue strips of the stomach musculature of the toad Rana marina were digested with trypsin and collagenase, isolated, and mounted under conditions 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) microelectrodes.

When external calcium was elevated to 100M or greater, an action potential could be elicited. 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 somatic conditions as low as 30M. A comparison of overshoot amplitude in 12 vs. 100M Na+ solutions (choline substitution for Na+ with Ca++ constant at 21mM) disclosed a small change of only 40% (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 at 10mM Ca++, 15mV at 50mM Ca++, and 13mV at 100mM Ca++. All other parameters measured were identical. 3) At high concentrations of Na+ and Sr++ (64-76M, 18mM TEA), the cells were sensitive to burst generation, with 100mM Na+ and 0.09M the 'plateau' in the range of 100 msec preceding the phase of maximum repolarization; prolonged repetitive discharge was common. The steady-state current-voltage relationship of the cells allowed assessment of input resistance. Input resistance was increased with [Ca++]o was raised and often exceeded 500MΩ, ranging as high as 3000MΩ; specific resistance often occurred above 30-100mΩ cm² and ranged as high as 150-1000mΩ cm². Specific membrane capacity was 1.320±0.33 μF/cm² (mean±S.D.). Supported by Nat’l. Fdnr March 24, 1974.

**100 CaO Na**
MEMBRANE STRUCTURE AND FUNCTION

GIANT neurons are ideal for studying the synthesis of membrane glycoproteins and their subsequent assembly into specific organelles. Injecting 3H-L-fucose or 3H-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 glycoproteins are first associated on membranes of the reticulum and Golgi and later become associated with other synaptic 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 NaB3H4. Treatment of R2 in situ with 0.01% trypsin 24 h after injection releases 3H-glycopeptides into the bath, indicating that glycosylation extends outward from R2's surface. R2's resting potential was normal throughout this mild digestion.

Thus, 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 L-fucose or N-acetylgalactosamine, an inhibitor. Thus, in cells injected under these conditions almost all of the incorporated radioactivity is present in this glycoprotein. Analyses of radioautographs showed a proportion of silver grains over vesicles in cells exposed to the inhibitor compared with untreated cells. To demonstrate the association of this component with the external membrane, a cell from R2 injected with treated with aminycin was fractionated in a sucrose gradient. A fraction enriched in vesicles, the cytoplasm from an injection site, was isolated and was found in a vesicle fraction characterized by electron microscopy. When cytoplasm from untreated cells was fractionated in this way, 3H-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 in this vesicle component. Further fractionation is necessary to determine whether specific types of vesicles contain unique glycoproteins.

NEUTRAL AMINO ACID TRANSPORT INTO ISOLATED BRAIN CAPILLARIES: EVIDENCE FOR POLARITY OF THE BLOOD-BRAIN BARRIER. A. Lorris Bet* and Gary W. Goldstein, Deps. of Neurology and Pediatrics, Univ. of California, San Francisco, CA 94143.

A different polarized somatic glycoprotein occurs by at least two distinct systems, a Na+-dependent A-system and a Na+-independent L-system. The neutral amino acid analogue of (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 αMeAIB and 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°C for various periods of time in the presence of L-leucine and amino acid influx 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. When the transcellular Na+ gradient was eliminated by preincubation with ouabain, the 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 capillaries, it is likely that they are located on the abluminal (brain side). Thus, brain capillary endothelial cells demonstrate a functional polarity for neutral amino acid transport.


Particle-free patches have been reported in freeze-fracture replicas of mouse and bovine ROS plasma membrane (Jan and Revel, J. Cell Biol. 67: 257-273, 1974; Krebs and Köhn, Exp. Eye Res. 25: 311-316, 1977), and have been observed by us in mouse, rat, and goldfish acclimated to about 20°C. Persistence of these patches in mouse ROS incubated at temperatures from 5 to 80°C raises doubts that they are 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 (Tillich and Kinsky, Exp. Eye Res. 1: 233-237, 1971) as a result of interaction with sterols present in exposed membranes (Kinsky, Ann. Rev. Physiol. 10: 119-142, 1970). Hubbell'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 as well. Mature discs have no patches or pits. The plasma membrane of the scotiod portion of the inner segment is densely packed 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.

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-(methyl-amino)isobutyric acid (αMeAIB) (Neuroph. 28:363, 1978). The technique is based on very widely used AIB in vivo studies of AIB and other aromatic amino acid transport out of the cell. We have constructed a brain ECF model in which the brain capillary was separated from the blood by a very thin tissue layer (30 μm) which was constructed. The rate constant for blood to brain ECF transport (Kb) 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 Kc obtained for rat cerebrum and rhesus monkey caudate nucleus and corpus callum were 1.8x10^-3, 3.4x10^-4, and 2.6x10^-5 min^-1, respectively. The capillary rate constant for AIB transport in the opposite direction (Kb) is brain ECF to blood and was measured from ventriculocisternal perfusion experiments with serial periventricular tissue sampling in the rhesus monkey. Kc was found to be less than 1x10^-5 min^-1. The brain cell rate constants for AIB influx were measured in vitro with brain slices of mouse cerebrum and cortex. The brain cell rate constants for AIB influx were measured in vitro with brain slices of mouse cerebrum and cortex. The brain cell rate constants were 1.4x10^-9, 8.4x10^-10, and 3.4x10^-10 min^-1, respectively. The cell influx rate constants for AIB transport out of the cells of the above tissues were calculated to be 4.3x10^-9, 1.0x10^-9, and 1.4x10^-9 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 this increase in capillary permeability to a brain region as small as 100 to 200 μm in diameter. In vivo autoradiographs demonstrate brain capillary disruption caused by a freeze lesion will be presented.
The specific binding of batrachotoxinin A-20α-paraJH-benzoate (H-BTX-B) - specific activity 12c 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 homogenate, specific binding was measured with a pellet assay as the difference in 3H-BTX-B binding in the presence and absence of a 100-fold excess of unlabeled H-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 Kd = 2 x 10^-8M. This binding is slowly reversible. The difference in 3H-BTX-B binding in the presence and absence of a membrane preparation lacking voltage sensitive sodium channels, has been characterized with respect to the apparent dissociation constant.

The specific binding of batrachotoxinin A-20α-paraJH-benzoate (H-BTX-B) - specific activity 12c 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 homogenate, specific binding was measured with a pellet assay as the difference in 3H-BTX-B binding in the presence and absence of a 100-fold excess of unlabeled H-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 Kd = 2 x 10^-8M. This binding is slowly reversible. The difference in 3H-BTX-B binding in the presence and absence of a membrane preparation lacking voltage sensitive sodium channels, has been characterized with respect to the apparent dissociation constant.
761 SOUND STIMULATION INCREASES POLYPHOSPHOINOSITIDE LABELING IN AN ACOUTERIC RECEPTOR. Patricia L. Kilian and Jochem Schacht. Kresse Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

The possible involvement of polyphosphoinositides in hearing processes was investigated in the auditory organs of the moth, Agrotis ypsilon. This organ was selected because of its morphological and electrophysiological simplicity. The sensory tine of this organ is the scoloparium, an array of 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 (60 kHz) of supra-threshold intensity for 30 min after an injection of 32P-orthophosphate. This stimulation led to a 50% increase of labeling of both phosphatidylinositol and phosphatidylserine. 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, pulsing tones trigger both generator and action potential while continuous tones lead to an adaptation of these potentials. This suggests that the increased labeling of polyphosphoinositides is associated with spike activity in this tissue.

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).


Antigenic components of neural membranes. S. P. Mahadik, A. Krenovsky*, P. Happle, and H. Krigman. Biological Sciences Research Center, Department of Biochemistry, and Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27514.

Membrane structure and function


A small molecular weight, anionic glycoprotein from brain synaptic membranes has previously been purified and found to bind L-glutamic acid with a high affinity (Kd 0.7-1.0M) (Michaelis, Biochem. Biophys. Res. Commun. 65, 1004, 1975). Recent studies employing sucrose-gradient ultracentrifugation revealed that this glutamate binding glycoprotein (GBP) tends to form multimers with molecular weights of 24,780, 40,600, 60,400 and 103,500. The specific binding activity is highest in the 40,600 and 60,400 M.W. form of the GBP. Pretreatment of this glycoprotein with 2-mercaptoethanol (10-4M) reduces the binding activity of the protein to a level that is comparable to that obtained from the total membrane fraction from rat brain cortex.

In sensory processes other than hearing since these lipids were released from normal animals were incubated with varying concentrations of lead. The introduction of lead was not inhibited by equivalent concentrations of Ca++. It is concluded that lead is tightly bound to the carrier, a finding consistent with the fact that this association interferes with the carrier mechanism of monosaccharide transport. (Supported by U.S.P.H.S. grants ES01104, NS11615 and HD03110).

764 ANTIGENIC COMPONENTS OF NEURAL MEMBRANES. S. P. Mahadik, A. Krenovsky*, P. Happle, and H. Krigman. Biological Sciences Research Center, Department of Biochemistry, and Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27514.

Membrane structure and function


Antigenic components of neural membranes. S. P. Mahadik, A. Krenovsky*, P. Happle, and H. Krigman. Biological Sciences Research Center, Department of Biochemistry, and Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27514.
CARBOHYDRATE-CONTAINING MACROMOLECULES. A MODEL FOR THE LOCATION OF BASIC MYELIN PROTEIN (MBP) 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

This work was done during the tenure of an IDA Fellowship.

Membranes and PE microvilli which may be related to the breakdown or composition of certain cell surface sugars on photoreceptor OS observations may reflect some basic change in the accessibility difference between control and RCS retinas is the absence of CB debris membranes. The lectin labeling on PE microvilli in both membranes of RCS retinas, whereas LC appears to label some of the 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 label uniformly 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 shows some degeneration well. In contrast, WGA and CB do not label the OS 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 the disc membranes of 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.

A MODEL FOR THE LOCATION OF BASIC MYELIN PROTEIN (MBP) AND PROTOLEPID (PL) ON LAMELLAR 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 on a water surface (e.g., BBA 311, 420). Lateral 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 MBP into artificial vesicles (e.g., Vahl, et al., PNAS 74, 1256) indicates 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 frozen for long periods of time causes swelling of the particles, suggesting that the PL alone cannot be responsible for the particles. Freeze fracture also indicates a structural link between particles (neurofilamentation) which must extend across both the main dense line and the intermediate line (Pinto da Silva and Hiller, 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 another membrane. Arguments of evidence and arguments arising from the swelling properties of myelin (e.g., Fimman, and Millington, J.B.C.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 IDA Fellowship.

EFFECT OF ACETAZOLAMIDE AND FUREOSMIDE ON CSF PRODUCTION BY CAT CHOROID PLEXUS IN SITU. James M. Melby* and Donal J. Reed* (SPON: I.W. Woodbury) Dept. of Pharmacology, Coll. 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, I. Physiol. London 227:127-139, 1972) was used. After a control steady-state period 2-1/2 hours or more, 3-5 kg cats either side were given either 30 mg/kg acetzolamide or 50 mg/kg fureosmide intravenously and measurements were made at 1/2 hr intervals for 2-1/2 hrs. Acetzolamide 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 0.11 ± 0.01 SEM after 2-1/2 hr. Fureosmide 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 0 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 acetzolamide was greater than that produced by fureosmide). The only statistically significant change in CP efflux ocurred at the end of the 2-1/2 hr period, produced by both drugs. Acetzolamide lowered CSF [K+] from 3.18 ± 0.15 SEM mEq/l to 2.38 ± 0.22 SEM mEq/l (P < 0.05), while fureosmide lowered [K+] from 3.18 ± 0.15 SEM mEq/l to 2.25 ± 0.22 SEM mEq/l (P < 0.05). The ability of the two drugs to reduce the flow of the CSF 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 12669 and GM 00153).
KINETICS may not be so prominent with the ions at high levels, but the uptake kinetics, which we have verified, fit the carrier model shown below. According to this model, the binding of the transport substrate is facilitated by the presence of ions; for example, Na ions; only free carriers (C) and fully loaded carriers (Na–C-GABA$^+$) cycle slowly, if at all, in the absence of external Na. We also found that uptake was not affected in cells isolated from young rats (Cotman et al., J. Neurochem. 23: 941, 1974). This suggests that carrier-mediated GABA uptake involves the transport of ions (e.g., Na, moving out with the GABA, which is a net flux at neutral pH).

outside GABA inside GABA

outside GABA inside GABA

earlier stages of exocytosis were captured using the rapid-freezing technique described by Martin et al. (J. Neurochem. 23: 155, 1975). By high-speed photography, we have observed the fusion of the carriers (amylocytines) from the horseshoe crab, Limulus polyphemus and used freeze-fracture and freeze-substitution techniques to prepare them for electron microscopy. The results show that the carriers (amylocytines) can be found in many different structures; for example, they are seen in both synaptic and non-synaptic regions of the nervous system. Some of the most interesting results are summarized in Table 1, which shows the number of carriers observed in different regions of the nervous system. The table indicates that the number of carriers observed in the synaptic regions is significantly higher than in the non-synaptic regions. This suggests that the carriers (amylocytines) play a more important role in synaptic transmission than in non-synaptic transmission. This finding is consistent with the hypothesis that the carriers (amylocytines) are involved in the regulation of synaptic transmission.
CROSS TALK BETWEEN BARE AND MYELINATED AXONS IN SPINAL ROOTS OF DYSTROPHIC MICE. Michael Rasmynsky. 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 improperly 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 thin fibers (Rasmynsky, Brain. 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 lumbar spinal roots of 129F1/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 recordings 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 ¾ 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 (Rasmynsky et al., Brain Res. 163:71,1978) 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 capacitative bare axon.

PHOTOLABELING OF SURFACE PROTEINS OF RAT BRAIN SYNAPTOSOMES. James S. Scheinman, Michael Bures, Mark Stiefel*, and Michael V. Yack. 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 inserted 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 atomic composition of these functional presynaptic proteins.

Whole rat brain synaptosomes were prepared by the procedure of Oudiet al. (J. Neurochem. 22:281, 1974) and photolyzed in the presence of 10μM of tritium labeled N-4-azido-2-nitrophenylglycine at 4°C for one hour. All experiments were run at pH 7.4 to ensure that the photolabel was completely cycled 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-dried, solubilized in sodium dodecyl sulfate (SDS), heated to 100°C and dialyzed. The resulting solubilized synaptosomal protein mixture was resolved into eighteen bands by SDS polyacrylamide gel electrophoresis. One-dimensional gel slices were solubilized and counted. Radioactivity was detected in slices corresponding to molecular weights of 125K, 112K, 92K, 66K, 54K, and 41K. 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 41K. Supported in part by the UMC Research Council.

ATPASE ACTIVITIES IN RETINAL PIGMENT EPITHELIUM AND CHOROID. Michael V. Riley*, Barry S. Windeler, Jennifer Bennett*, and Ellen M. Yates*. Institute of Biological Sciences, Oakland University, Rochester, Michigan 48063.

The Na⁺-, K⁺-, and Na⁺-K⁺- dependent ATPase activities of retinal pigment epithelium-choroid tissue from grassfrog, bullfrog and rabbit have been measured. In the frogs, Na⁺-ATPase activity was between 1 and 2 μmoles P_i liberated hr⁻¹ mg⁻¹ protein, while Na⁺-K⁺ ATPase and HCO₃⁻ 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 μM calcium, while HCO₃⁻ 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. HCO₃⁻ ATPase and Na⁺-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.

PHOTORECEPTOR CELLS OF DROSOPHILA. Rudolf H. Schin* (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 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 biological significance of this type of junction is still poorly understood. Supported by NSF BNS77-18647 and NIH EY00033 granted to W.T. Pak.
The use of antibody + complement to gain access to the interior of prestataptic terminals. Erik S. Schweitzer and Nordec P. Blaustein, Department of Physiology and Biophysics, Washington University Medical School, St. Louis, MO 63110.

Treatment of synaptosomes with antibody 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 the 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°C. When the incubation medium is warmed to 30°C after a 20 minute incubation at 0°C, K release is complete in less than 5 minutes. This latter arg can be carried out in a medium containing sub-micromolar Ca'2+.

The pemp-selectivity of the complement-induced pores is consistent with a pore size of 25 Å, as has been suggested by Michaela and Meyer (Biophys. J. 21: 125a, 1978). The pores are large enough to allow the rapid release of intraterminal 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 synaptonsomes.

This treatment may allow the manipulation of the intraterminal ion and metabolite composition without the loss of microvesicles or subcellular organelles, such as synaptic vesicles, microtubules, endoplasmic reticulum, and mitochondria. [Supported by USPHS grant NS-04882.]

Localization of rapid blood-Csf barrier Na-K ATPase. Quinn R. Smith and Carol F. Tohn (Spon. W. Stevens, Dept. of Pharmacology, Univ. of Utah Col. Med., Salt Lake City, Utah 84132)

Two properties of ouabain, the bipsitic effect on Na-K ATPase and its low permeativity, were utilized to investigate the localization of Na-K ATPase in the blood-CSF barrier. Adult rats were injected i.p. with ouabain at a dose of either 0.1 or 10 mg/kg (or vehicle) and sacrificed 1 h later for analysis of Na and K. The extracellular fluid space and resadigl electrolyte volume were measured with tritiated inulin and 31Cl-tagged-RCB, 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 mmol/kg H2O and cell [Na] fell by 45% to 25 mmol/kg. The cerebral cortical cell [K]/[Na] increased, but not significantly. Although plasma [K] increased by 101% to 9.41 mmol/L and plasma [Na] decreased by 7% to 140 mmol/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 effect in 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 0.1 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]


Myelin-associated partial fraction (R) and purified myelin (M) isolated from the white matter of rat brain were phosphorylated by the endogenous (Mg2+-supported) protein kinase (ePK). With either R or M purificaiton, the following results were obtained: The esterase activity of the blood-CSF barrier. Adult rats were injected i.p. with ouabain at a dose of either 0.1 or 10 mg/kg (or vehicle) and sacrificed 1 h later for analysis of Na and K. The extracellular fluid space and resadigl electrolyte volume were measured with tritiated inulin and 31Cl-tagged-RCB, 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 mmol/kg H2O and cell [Na] fell by 45% to 25 mmol/kg. The cerebral cortical cell [K]/[Na] increased, but not significantly. Although plasma [K] increased by 101% to 9.41 mmol/L and plasma [Na] decreased by 7% to 140 mmol/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 effect in 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 0.1 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]

The large subunits of two different Na,K-ATPases from mammalian brain can be separated by polyacrylamide gel electrophoresis in SDS. We have identified two bands differing by 200 K in apparent molecular weight. Both bands are phosphorylated by γ-[32P]ATP in the presence of Na⁺ and are dephosphorylated in the presence of K⁺. The bands cannot be phosphorylated by either the synthetic peptides of cyclic AMP or ATP, and in their reactivity to N-ethyl maleimide. In the rat, there are two Na,K-ATPase activities in brain with dramatically different classes of Na,K-ATPase; 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 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 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-ATPase found in kidney, cardiac muscle, skeletal muscle, and cultured sympathetic neurons. Both species are found together in white and gray matter, in retina, and in sympathetic ganglia. 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 both, but not all, classes of neurons. The possibility that the two ATPases modulate synaptic activity in different ways deserves further investigation.

PEERMEABILITY OF THE FROG PERINEURIUM UNDER CONDITIONS OF STRETCH AND HYPERTONICITY. Amanda Weaversey*, Robert E. Taylor and Stanley J. Rapoport. NINDS, NIH, Bethesda, Md. 20014

The permeability to 32P-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 perineural 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 32P-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 perineural surface area. Mean permeability was calculated to be 5.6±0.27 (S.E.M., n=4)X10⁻⁶ cm² sec⁻¹. Stretch of the perineurium by 10% from rest length reversibly increased perineurial permeability to 32P-sucrose by a factor of 3, but the increase was not fully reversible when the preparation was returned to rest length. The perineural 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 NaCl. The addition of another NaCl to this bath 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 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 were conducted using a 32P-sucrose bath for the first time, the diffusional restriction that the perineurium places on exchange between the endoneurial space and general extracellular fluid space of the nerve, and indicate that stretch or hypertonicity can reduce this restriction.

IDENTIFICATION OF LECTIN BINDING POLYPEPTIDES IN MICROSONAL FRACTIONS OF FIG CEREBELLAR CORTEX. John G. Wood and Francis J. 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., 63:541-547, 1974; Brain Res., 116-117, 1976) which function 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 isopropanol 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 (Con A) and gel, 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 Agarose gel CB and WGA and CB were very similar, a result in accord with our cytochemical results (J. Cell Biol., 25:117a, 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 cell stemrites while CB does not (Con A, Wood and Byrd, 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 helpful in identifying a wider range of reactivity and gel labeling studies. Supported by NS-12590 and the Sloan Foundation (JOM)
CLOSE ASSOCIATION OF SELECTIVE ACID HYDROLASES WITH RAT CNS MYELIN. Shuichi Yamaguchi, Eizuke 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-glucosamine 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, GM1-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, galactosylceramidase, sphingomyelin, GM1-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-03536, and HD-01799 from the U.S.P.H.S.)


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 excitable muscles of the fresh water crustaceans Xiphocaris elongatus and Athys 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 Stenus huspidus was used for this purpose. The reagents used were N-ethylmaleimide (NEM), maleimide and 4-cyclopentene-1,3-dione (4-CPD). Exposure of Stenus muscle to these reagents (2mM, 5-10 min) induces action potentials with low membrane accommodation. 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-cyclopentadione, the saturated analog of 4-CPD, has no effect. The spikes are not affected by tetradotoxin (50µM) but are reversibly blocked by Me2+ (50µM), suggesting they are partially due to Ca2+ 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 chloethers 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

Psychomotor stimulant drugs which serve as dopamine agonists have been shown to potentiate conditioned reinforcement (Hill, 1972; Hill and Robbins, 1975). However, the blockade of dopaminergic neurons in the establishment of conditioned reinforcement has not been investigated. As a direct test of this possibility, the present experiment explored the effect of dopamine receptor blocker pimozide on the establishment of conditioned reinforcement in which a tone was paired with food reward. The experiment design consisted of five groups (two 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, the lever absent was the tone lever choice 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 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 min after each session of phase two and during phase three. This group showed a shift in preference for the tone lever thus showing that the state dependent learning group failed to show conditioning to the tone because of a drug-induced motor impairment. These results implicate the involvement of learning processes which underlies the establishment of conditioned reinforcement.


BIOCHEMICAL AND BEHAVIORAL EFFECTS OF STREPTOVITCIN A IN MICE. C.A. Bream and B.K. Agrawal. 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. In the current study, small 18 mg dosage has been utilized to localize studies in the goldfish. In experiments with mice (Kichinbaum et al, Brain Res. 101:171, 1976) we found memory loss following bilateral intracerebral injections of cyclobarbide (CMD, 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 CMD 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 streptovitcin 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 was seen after injections immediately after or 1 h following training, while a 6 h delay was less effective, a result consistent with the well-established 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 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.)


Results obtained in a recent experiment in this laboratory drew attention to the possibility of feeding additional 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. By an important 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 experiment was 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 supported previous findings of early latency associative changes in MGB, and are consistent with the idea that such changes are most likely to occur under the medially 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.
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. Il Buzy-Cartwright and 
C. Reyes Vázquez*. Depto. de Fisiología, Div. de Investiga- 
cción, Fac. de Medicina, UNAM. México 20, D.F., México.

Different observations have shown the involvement of catechol-
amines 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 sessions, under pentabarbitol anesthesia, was injected bilateral injections of 6-hydroxy-
dopamine (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) significantly 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 test comparisons of 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 micro-
injections of NaCl or NaCl on 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 with NaCl showed a slight increase of lever pressing in the suppression condition, while NaCl produced a decrement of the lever pressing. This depression 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.

IMPAIRED MOTOR MEMORY AND INTEGMENT MOTOR SKILLS 
ACQUISITION IN ANTEROGRADE AMNESIA. Neal Cohen and 
Larry R. Squire*. University of California, School of Medicine, 
UCSD, School of Medicine, La Jolla, CA 92037.

Studies of human amnesia have reported that acquisi-
tion of perceptual-motor skills is spared in patients who otherwise are deficient in committing new informa-
tion to long-term memory. In normal subjects, rhin-
thetic (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 thera-
py (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 inter-
val (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 en-
job's a special neurological status. Possible differ-
ences between motor skills acquisition and motor memory will be discussed.

COMPARING NEURAL PLASTICITY IN THE HIPPOCAMPUS DURING CLASSICAL 
CONDITIONING OF THE RABBIT NICOTINATING MEMBRANE RESPONSE TO LIGHT 

Unit activity was recorded from the CA1 area of dorsal hippo-
campus in New Zealand White rabbits (Phryneus cuniculus) during conditioning. The conditioning paradigm involved using a corneal air puff unconditioned stimulus (UCS) to classically condition nicotine-evoked membrane movement to the presentation of either a tone or a light conditioned stimulus (CS). Twelve rabbit were divided equally into 3 stimulus presentation groups. In the first group the stimulus presentations consisted of pair-
ing 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, unperturbed presentations of 
either the light or air puff. For all 3 stimulus presentation 
groups, the CS was presented for 300 msec and the UCS was pre-
sented for 100 msec. For the paired presentations, the CS and UCS were overlapping and cotemimating. 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.

ATROPINE INJECTIONS IN THE ANTERIOR AND POSTERIOR CAUDATE NUCLEI: 
EFFECTS ON PASSIVE AVOIDANCE. Sara E. Cruz-Horta, Flavio A. Lopez-Morilla, Rufino A. Sánchez-Ayala and Carlos E. A. 

Cholinergic blockade of the caudate nucleus (CN) produces sig-
nificant deficits in learned behaviors. Further, differential 
impairments are found, in active avoidance, after dorsal or ven-
tral blockade of the head of the CN.

The present results deal with the effects of atropine injec-
tions in different areas of the CN.

Rats were implanted with cannulae in the anterior or post-
erior 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 significant impairment in reten-
tion ability.

These results further support the hypotheses that: a) a cholin-
ergic mechanism is involved in the CN, in learned perfor-
mance, and b) the caudate nucleus is not functionally homogeneous.
795 AMNESIA IN CHICKS AND RATS INDUCED BY 3,4-DEHYDRO-DL-PROLINE.
Joel S. Davis and Arthur Cherkisin.
Psychology Research Laboratory, VA Hospital, Sepulveda, CA 91343.

Intraventricular injection of L-proline (L-PR0; 6.0 µmol/chick) or its analog baikalin (4.5 dehydro-DL-πpecic acid; 1.5 µmol/chick) induces retroactive amnesia without brain seizure or isoelectric activity. D-PR0 is non-amnestic. We now report that 3,4-dehydro-DL-proline (D-PR0), an analog of PR0 and baikalin, has amnestic properties. We injected chicks intracerebrally with 10 µL/hemisphere of 150 mM L-PR0 or D-PR0, or 75 mM D-PR0, 1 min after one-trial training to suppress the peck response to a stainless steel bead. Amnesia was conditioned by coating the bead with an aversive liquid (methyl anthranilate) immediately prior to training. Retention of avoidance was tested 24 hr after training using the uncoated bead; reduced avoidance scores and increased peck scores indicate impaired memory.

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Avoidance Score (%)</th>
<th>Peck Score (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-PR0</td>
<td>47.3</td>
<td>1291.5</td>
</tr>
<tr>
<td>D-PR0</td>
<td>49.2</td>
<td>2483.3</td>
</tr>
</tbody>
</table>

The data in Table 1 demonstrate that D-PR0, an effective amnestic agent at a dose (3 µmol) below the amnestic dose of L-PR0 (6 µmol); for DHP and L-PR0 at 3.0 µmol, the avoidance scores (p<0.0001; x̄±SEM) to lick a tube containing water. After reaching a predetermined latency criterion, rats were given a single 3 sec, 3 mA footshock and then tested for retention of the footshock experience 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 with stainless steel cannulae chronically implanted into the amygdala with noradrenaline (NE). Injections of NE (32 µg) into the hippocampus of Wistar male, 50-65 days, old were injected through implanted cannulae with 5 µL of 150 mM D-PR0 (1.5 µmol) 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.

797 HOREPINEPHRINE INJECTIONS INTO AMYGDALA IMPAIR PASSIVE-AVOIDANCE LEARNING. Maureen A. Fix and Raymond P. Kemner.
Dept. Psychol., Univ. of Utah, Salt Lake City, UT 84112.

Water-deprived rats were trained to enter a goal box in order to lick a tube containing water. After reaching a predetermined latency criterion, rats were given a single 3 sec, 3 mA footshock and then tested for retention of the footshock experience 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 with saline or DHP and L-PR0 at 30 min or 60 min. Previous scores for 6.0 µmol of L- and D-PR0 are tabulated for reference. No seizure spiking or isoelectric activity from chs were found in EEG recordings during L- and D-PR0 conditions resembling the behavioral paradigm. We injected DHP intracranially into mice 1 min or 60 min after one trial passive avoidance training. Mice 30-50 days old were injected through implanted cannulae with 5 µL of 150 mM D-PR0 (1.5 µmol) 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.

798 CHANGES IN DENDRITIC SPINES OF THE DENTATE MOLARULAR LAYER DURING CONDITIONING.
Rayna Fischer and A. Van Harreelen

Following a classical conditioning paradigm we observed an enhancement of dendritic spines at the projection site of the perforant path in the dentate molecular layer (Neurosci. Abstr. 1998). Present experiments were aimed at examining the effect of conditioning and pseudoinactivation on spine dimensions in the dentate molecular layer. Forty-seven mice (18 g) had their 1st day 50 pellets (45 mg) available in adjacent experimental cages. On the third day animals were assigned either to the conditioning, pseudoinactivation or control procedures. In single 2 hr sessions followed by a pellet were presented during conditioning and 50 trials of a random presentation of tone and pellet were given during pseudoinactivation. Controls only 2 hr 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 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 (125; p<0.001) and distal (137; p<0.001) layer compared to non-inactivated controls. In pseudoinactivated animals the area of dendritic 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. The control group had spine dimensions of all spine dimensions in the middle and distal third. However, spines in the proximal third of "starved controls" were significantly smaller (65; p<0.005) than of non-inactivated controls. Thus, 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 52724.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Avoidance Score (%)</th>
<th>Peck Score (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-PR0</td>
<td>47.3</td>
<td>1291.5</td>
</tr>
<tr>
<td>D-PR0</td>
<td>49.2</td>
<td>2483.3</td>
</tr>
</tbody>
</table>

**Figure 1**

- Panel (a): Changes in dendritic spines as a function of time and treatment condition.
- Panel (b): Changes in dendritic spine area as a function of time and treatment condition.

**Mean values ± standard errors of the average spine area in arbitrary units in different parts of the dentate molecular layer.**
801

DEVELOPMENT OF DISCRIMINATIVE UNIT ACTIVITY IN CICLADIC CORTEX AND ANTEROVENTRAL THALAMUS DURING CONDITIONING AND OVERTRAINING IN RABBITS: Michael Gabriel, Kent Foutser, and Edward Chona. Dept. Psychol., Univ. Texas, Austin, TX 78712.

The function of opioid peptides in brain regions not generally associated with pain processing remains largely underexplored. 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 consistent with other reports 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 effect could be facilitated by post-training administration of opiate agents into the amygdala of rats on retention of passive avoidance behavior.

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 the antagonist naloxone into the amygdala immediately after training significantly increased retention. This effect was likewise dose-dependent and was demonstrated in all of the doses used. The naloxone and levorphanol administration into the amygdala immediately after training blocked the retention deficit produced by opiate agonist administration. Finally, injections of the opiate agents comparable to those which significantly altered retention when injected into the amygdala did not significantly alter retention when injected into the cortex. 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 K02 HD00118 (NIMH) to B.S.K.

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 supraspatic deaucssation (DSO) is apparently necessary for transfer of pattern discrimination learning in adult chicks. The DSO 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 (+, -) 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 retrained with the trained eye demonstrated excellent retention of the discrimination task. In marked contrast, the animals tested for interocular transfer showed 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 trained 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 NSF GRANT # MH-03371.

RIGHT HEMISPHERE SUPERIORITY IN THE PERCEPTION OF FACIAL EMOTION. Edward C. Hasick*, Francis J. Pirozallo, and Betty*. 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 & Niedenthal, 1973). The present study provides clinical neurological evidence for a diminished capacity to respond to emotionally-charged stimuli in patients with right hemisphere brain damage (Wechsler, 1973). While facial recognition has been reliably demonstrated as a right hemisphere specialized skill (Pirozallo & 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 latency 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 facial emotion perception, and cast doubt on the alternative hypothesis that a constant attentional bias to a given visual field mediates laterality effects. These data would also suggest that clinical observations that patients with right hemisphere disease have difficulty discriminating and comprehending emotionally-charged stimuli (Hedden, Shoes & Watson, 1975; Tucker, Watson & Heilman, 1977).

EEG DEPRESSION IS NOT ESSENTIAL FOR L-PROLINE INDUCED RETROGRADE AMNESIA. L. K. Gerbrandt* and J. L. Davis. Dept. Psychol., CBM, Northridge, CA 91324 and Psychology Research Laboratory, VA Hospital, Sepulveda, CA 91343.

Previous experiments (Gerbrandt et al., 1977) have shown EEG amplitudes at multiple units of integration within 60 sec after injection of l-proline (L-P.) under amnestic levels of l-proline (L-P.). Since activity like spreading depression (S.D.) has been shown to cause amnesia (Buresova & Bures, 1969), it was further tested whether amnesia produced by L-P. includes a S.D. induced component. The objective of these experiments is to compare electroencephalographic and behavioral effects of L-P. with and without a criterion of KCl sufficient to produce EEG depression similar to those observed after L-P. injection. Chicks (n=4), 4±12 hrs old, were implanted with bipolar electrodes in the cortex and thalamus. The following day, after 15-min adaptation and baseline period, chicks were trained with methyl anthranilate (MaA) to mimic interactions of training and testing stimuli. The MaA was in injected intracerebrally with 10 ml/hemisphere of 300 mM L-P (N=6), or 250 mM KCl (N=6). EEG was recorded for 11 min after injection. The degree of suppression of EEG activity. The preliminary experiments indicated it would yield a reliable EEG depression comparable to that by 300 mM L-P. None of the injections produced motor activity or seizures. The preliminary experiments indicated it would yield a reliable EEG depression comparable to that by 300 mM L-P. None of the injections produced motor activity or seizures.

To test whether 250 mM KCl induces a S.D. related amnesia, we injected chicks intracerebrally with 10 ml/hemisphere of 300 mM L-P (N=90), 300 mM D-P (N=85), or 250 mM KCl (N=87) 1 min after a 10 sec one-trial training of the chicks to suppress their "instinct" peck response to a small, shiny bead. Peck suppression, with avoidance of the normally attractive bead, was conditioned by coating the bead with an aversive (MaA). Retention of the avoidance was measured 24 hr after injection of the MaA-coated bead; increasing pecking indicated impaired memory retention. Results confirm the amnesic effects of L-P. relative to D-P. (p<.005), and to KCl (p<.005). KCl did not differ significantly in its amnesic effects from D-P. (p>.10).

These results indicate L-P. induced amnesia cannot be duplicated by another method of inducing EEG depression by L-P. injected alone. Therefore, these data support the Van Harrevel & Filipova theory that L-P. 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.

NEURAL ACTIVITY RECORDED IN THE ABDCUCUS AND OCULOMOTOR NUCLEI DURING NEUTRAL AND EMOTIONAL CONDITIONING IN THE RABBIT. Comparable durations and amounts of depression were found for KCl and L-P. to test whether 250 mM KCl induces a S.D. related amnesia, we injected chicks intracerebrally with 10 ml/hemisphere of 300 mM L-P (N=90), 300 mM D-P (N=85), or 250 mM KCl (N=87) 1 min after a 10 sec one-trial training of the chicks to suppress their "instinct" peck response to a small, shiny bead. Peck suppression, with avoidance of the normally attractive bead, was conditioned by coating the bead with an aversive (MaA). Retention of the avoidance was measured 24 hr after injection of the MaA-coated bead; increasing pecking indicated impaired memory retention. Results confirm the amnesic effects of L-P. relative to D-P. (p<.005), and to KCl (p<.005). KCl did not differ significantly in its amnesic effects from D-P. (p>.10).

These results indicate L-P. induced amnesia cannot be duplicated by another method of inducing EEG depression by L-P. injected alone. Therefore, these data support the Van Harrevel & Filipova theory that L-P. 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.

606 NEURAL ACTIVITY RECORDED IN THE ABDCUCUS AND OCULOMOTOR NUCLEI DURING NEUTRAL AND EMOTIONAL CONDITIONING IN THE RABBIT. Comparable durations and amounts of depression were found for KCl and L-P. to test whether 250 mM KCl induces a S.D. related amnesia, we injected chicks intracerebrally with 10 ml/hemisphere of 300 mM L-P (N=90), 300 mM D-P (N=85), or 250 mM KCl (N=87) 1 min after a 10 sec one-trial training of the chicks to suppress their "instinct" peck response to a small, shiny bead. Peck suppression, with avoidance of the normally attractive bead, was conditioned by coating the bead with an aversive (MaA). Retention of the avoidance was measured 24 hr after injection of the MaA-coated bead; increasing pecking indicated impaired memory retention. Results confirm the amnesic effects of L-P. relative to D-P. (p<.005), and to KCl (p<.005). KCl did not differ significantly in its amnesic effects from D-P. (p>.10).

These results indicate L-P. induced amnesia cannot be duplicated by another method of inducing EEG depression by L-P. injected alone. Therefore, these data support the Van Harrevel & Filipova theory that L-P. 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.
FOUR MEMORY CHANNELS FOR SHUTTLE-BOX LEARNING IN THE RAT. Ivan Izquierdo, Deusa A. Vendite*, and Elaine Elisabetsky*. Dept. Bio-
Instr. Bio-Sciences, 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 (DC-DC), in which the two sessions were paired (i.e., given contiguously) on every trial, regard-
less of whether the animals made shuttle responses to the buzzer or not; b) avoidance conditioning (DP-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. On all trials, both groups of rats were given 50 trials in both prepulse and test administration sessions consisting of 50 trials at 10-40 sec intertrial intervals. One or seven days after training the animals were sub-
mixed to a retraining session using either of these two paradigms, which had been trained, or the other one. Thus, it was in-
vestigated 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 chan-
cels could be tested: DP-DF, DC-DC, DP-DC, and DC-DF. In untre-
ated animals, only the first three channels were manifest. The DP-
DF 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 i.p. 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 investi-
gated. The DP-DF channel was insensitive to any of the three agents. 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. Fin-
ally, nicotine caused "unmasked" prevention of DP-DC conditioning. The effect on the four channels of immediate post-training hippocam-
ppal spreading depression was also investigated. This was pro-
duced by applying agar crystals onto the right anterior lateral and posterior planted cannulae, and was monitored electrophoretically. Control rats had the cannulae but received no KCl. Hippocampal spreading depression in the treated group lasted for at least 3-5 h. It had no ef-
fect upon operation of the DP-DF or DC-DC channels, but it en-
hanced that of the other two channels (DP-DF and DP-DF). These data do not support the hypothesis that integrative hip-
occampal function is in any way essential for memory consolid-
ation, and suggest instead a role for this structure in the pro-
cessing and sampling out of recently acquired information.

THE CENTRAL NUCLEUS OF THE AMYGDALA: BULBAR PROJECTION AND IN-
VOLVEMENT IN HEART RATE CONDITIONING. Bruce S. Kapp, Robert C. Fryxelger*, and Michela Gallagher, Dept. of Psychology, and
James S. Schaeber, Dept. of Anatomy, University of Vermont, Burlington, Vermont.
As a part of an effort to assess the involvement of specific amygdala systems in the development of heart rate conditioning, bilaterally lesioned were made in the dorsomedial amygdala of eight New Zealand rabbits, resulting in damage to the central nucleus and proximal fibers of passage. Two weeks following surgery the subjects received 15 presentations of the CS alone (CS+; 4.5 sec; 6.5 sec followed by shock) followed by the CS-
inten-
ting trials. The US was a 0.5 sec 2.0 mA eyeld shock coincident with the offset of the CS. The lesioned animals were signifi-
cantly impaired in the development of heart rate acceleration as compared to unoperated and operated control animals (p<.02). Since the conditioned cardiac response is mediated by the vagus nerves in the rabbit (Fredericks et al., 1976), the possibility was examined that a direct projection exists from the amygdala to the dorsomedial medulla, a region subserving cardiovascular regulation and containing cells of origin of vagal cardioinhibitory fibers in this species. Bilateral and uni-
lateral injections of horseradish peroxidase (HRP; 0.05-0.25 μl) were placed into the reticular thalamic nucleus or the medial part 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. Injections were also placed into the amygdala were sectioned at 40 micra and processed using benzidene as a chromogen. HRP-labelled amygdala neurons were found to be restricted to the central nucleus, the lateral amygdaloid nucleus, and the rostral hypothalamus. We are now using autoradiographic and anterograde HRP tech-
niques 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 deficit in the acquisition of conditioned cardiac responses, and (2) a cutaneous area of this zone of the projectio appears to be within which subserves cardio-
vascular reflexes and contains cells of origin of cardio-

Inhibitory fibers.

Supported by NIH Grant KOZ MH00118 and by a UVM Research Develop-
ment Award.

MORPHINE AND WALNOXENE ALTERNATE MEMORY IN THE RAT. Robert A. Jensen, Joe L. Martinez, Jr., Rita B. Messing, Vina Spiehler*; Beatriz J. Vazquez, Bernard Soumizu-Mourat*, K. C. Liang, and James L. McGeagh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA, 92717, U.S.A.
The effects of physical and chemical probes of the nucleus accumbens (NAc) on memory for contextual fear conditioning were investigated. 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 foot shock. Immediately after training, the rats received an i.p. injection of either Mor or NAc. NAc was administered in divided doses, 30 min apart, of Sal, 3.0, 10.0, or 30.0 mg/kg. NAc facilitated retention measured 24 hours later. Rats that received 1.0 mg/kg NAc showed significant facilitation of retention compared to controls with either a 500 μA (F(2,10; df=1, p<0.01) or a 750 μA footshock (F(2,10; df=1, p<0.01). Rats that received Mor, trained using a 750 μA shock, showed significant amnesia with both the 1.0 mg/kg dose (F(2;6, df=1, p<0.02) and the 3.0 mg/kg dose (F(2;6, df=1, p<0.01).
To test the receptor specificity of the effects, the capacity of Mor to attenuate the facilitatory effect of NAc was examined. Rats were trained as before with a 500 μA foot shock, and were given divided doses of either Sal, NAc (1.0 mg/kg), or a mixture containing both Mor (30.0 mg/kg) and NAc (1.0 mg/kg). All rats re-
ceived 2 injections 30 min apart. Rats that received NAc showed significant facilitation of retention (F(2;4, df=1, p<0.05). However, Mor blocked the facilitatory effect of NAc since animals that received the mixture differed significantly from the NAc alone group (F(2;4, df=1, p<0.02), but not from the controls (F(2;4, df=1, p<0.10).
The effect of intraventricular administration of Mor was then studied. Indwelling cannulae were stereotaxically implanted above the lateral ventricle, following the method of "mouse 'brain implant'". 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 caused significant amnesia (F(2;16, p<0.00469, df=1, p<0.05). These results suggest that the effects are mediated by central opiate receptors having a memory modulatory function. (Supported by USPHS grants MH 27526 and AG 04969 (J.L.McG.), Postdoctoral Fellowship 5T32 (R.A.J.), and a grant from the McKnight Foundation (J.L.McG.).

ETHANOL-INDUCED FACILITATION OF INHIBITORY AVOIDANCE PERFORMANCE IN MICE. K. D. Malcolm, R. E. Lovett* and K. L. Alkana, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.
It has been suggested that consolidation decrements may under-
ly 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 inhibi-
tory 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 (155 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 - 20 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 in-
creased 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 also did not significantly differ when compared to saline controls, suggesting that the ethanol injection was not aversive. In contrast to post-training treatment, injection of ethanol 24 h prior to testing did not result in ethanol-induced retention. The present findings do not support the consolidation hypothesis of ethanol's acute amnestic effects. These findings agree with the recent demonstration that post-
training administration of ethanol enhances memory storage. Further studies are necessary to replicate and extend these preliminary findings.
PERIPHERAL 6-HYDROXYDOPAMINE (6-OHDA) ALLOWS THE FACILITATORY EFFICACY OF AMPHETAMINE ON MEMORY. Joe L. Martinis, Jr., Robert A. Johnston, J. Robert Weisman, J. Santiago Vaquero, Bernard E. Poirier-Mourat, Debora Geddes*, K. C. Liang*, and James L. McGaugh, Department of Psychology, School of Biological Sciences, University of California, CA 92117.

We investigated, (1) the effects of peripherally administered 6-OHDA on acquisition of an inhibitory avoidance task, and (2) the effects of d-amphetamine (DAH) on learning and retention in rats sympathectomized with 6-OHDA.

Male Ash Sprague-Dawley rats (90 days old) were trained in a conditioned inhibitory avoidance task. Acquisition task training involved receiving 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 mA, 1 sec footshock. A retention test was given 24 hours later with 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 by approximately 25% of control and 100 mg/kg to 7% of control. There was no significant correlation between NE depletion of individual animals and their retention scores.

Our results showed that at 500 mg/kg 6-OHDA produced a significant retention deficit (t = 155.5, p < .05). Performance on the training task 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 mA footshock. Immediately following training rats received either no injection, SAL, 0.25, 1.0, or 4.0 mg/kg MPH, i.p. A 4.0 mg/kg dose of MPH 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 MPH was decreased to 0.25 mg/kg 6-OHDA, [t (36) = 1.36, 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 synaptic learning in memory and suggest that part of the action of amphetamine may be centrally mediated.

[Supported by USPHS grants #195256, #00469 (JLMG); Postdoctoral Fellowships #15429 (JLM Jr.), #15538 (RAJ); BNS 76-17370 (JLMG); and the McKnight Foundation (JLMG)].
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 prenatally exposed rats was focused on 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 postnatal 0, 10, 100, and 200 rads of Co60 radiation on: 1) differences in postnatal growth rates between control and irradiated offspring to differentiate transient developmental delay from permanent abnormal development, 2) development of reflexes and neurovascular 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 in the first 6 weeks after birth in the 100R offspring. According to the neurological evaluations, the postnatal development of reflexes and the maturation of neuro muscular coordination were 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 capacities from 1 to 2 years after birth. However, significant differences have been observed in visual discrimination reversal learning between control and 100R 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).

ASYMMETRICAL STATE LEARNING DEPENDENT PRODUCTION LIFE AND PHYSOSTIGMINE IN RATS. B. L. Ricketts (SPON: T. M. Shih), U.S. Army Biomedical Laboratory, Ft. APH, MD 21210.

In two separate studies using rats, 6.0 mg/kg of benactyzine or 0.6 mg/kg of physostigmine were tested for state dependent production learning 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 perturbation of the absolute level of cholinergic activity, regardless of direction, is more critical to retrieval of information during than during testing. Furthermore, the perturbation in itself does not disrupt information storage and retrieval mechanisms when those changes which existed during training are re-integrated during testing.

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. Assembly formation, reconfiguration, and re-assembly appear to function in brain function in that they could: maintain reverberatory firing activity for periods of the order of a second, provide stabilizing against local fluctuation, provide statistical reliability, and 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 Kauts, 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 history (RSH)). 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 spin-dendritic pyramidal cells found in the somatosensory cortex and of the hippocampus, 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 dendroedendritic 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 electro physiologically functional assemblies makes the effort worthwhile.

Immunohistochemical and double labeling experiments were used to determine that there was a transient increase in the concentration in the cerebral cortex and amygdala of two proteins, whose metabolism is markedly influenced by behavior, that were found only in the extracellular fluid (ECF) of the cerebral cortex and amygdala. These proteins were identified as a marker for the learning of new behaviors. They were found to be extracellular and responsive to the process of learning. The results also suggest that these proteins are involved in the process of learning and memory consolidation.

AFTER POSITRON EMISSION TOMOGRAPHY OF CORTEX AMYGDALA AND OBJECT CARD PHOTOCOUPLES* Spiegler* and Mortimer Mishkin. NIH, Bethesda, MD 20014.

On a test of one-trial learning of stimulus-reward association, monkeys showed marked decrement in learning of either the anterior or posterior temporal cortex (area TE) or the amygdala (A). By contrast, they showed only mild loss after lesions of either of the temporal cortex (area TEO) or the fusiform-hippocampal gyrus and hippocampus (FHB). Preoperatively, the monkeys had been trained to associate the reward value of an object on the basis of a single acquisition trial in which the object covered either a 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 11:1100, 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 separately, 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 relearning the objects' reward values and not by other strategies. On reaching the preoperative criterion, the monkeys received one of the four bilateral temporal cortex lesions (TE, A, or FHB) 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 first stage of this pathway. A previous study (Mishkin & Oubre, Neurosci. Abs. 2: 1127, 1976) showed that damage to TE, but not to other temporal lobes structures (TEO, A) was due to this performance on a one-trial learning test of object recognition as distinguished from object-reward association. Presumably, the impairment after TE lesions is the present case the same basic recognition disorder. The impairment after amygdala lesions, however, not being attributable to a recognition disorder, appears to reflect instead a disorder of memory consolidation. The results thus point to object recognition and object-reward association as processes that depend in part on the sequential participation of area TE and the amygdala.


Recently Bloch and his colleagues (Brain Res. 49: 367-369, 1973) have observed that a brief (2-4 hr) period of REM sleep deprivation (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 REM sleep 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 occupation 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 PSD, which procedure also fails to produce amnesia. We conclude that in mice PS immediately after eversive 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. 19:625-646, 1977).

EVIDENCE FOR THE SEQUENTIAL PARTICIPATION OF INFERIOR TEMPORAL CORTEX AND AMYgdALA IN STIMULUS-REWARD LEARNING. Brenda J. Spiegler* and Mortimer Mishkin. NIH, Bethesda, MD 20014.

On a test of one-trial learning of stimulus-reward association, monkeys showed marked decrement in learning of either the anterior or posterior temporal cortex (area TE) or the amygdala (A). By contrast, they showed only mild loss after lesions of either of the temporal cortex (area TEO) or the fusiform-hippocampal gyrus and hippocampus (FHB). Preoperatively, the monkeys had been trained to associate the reward value of an object on the basis of a single acquisition trial in which the object covered either a 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 11:1100, 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 separately, 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 relearning 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 FHB) 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 first stage of 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) was due to this performance on a one-trial learning test of object recognition as distinguished from object-reward association. Presumably, the impairment after TE lesions is the present case the same basic recognition disorder. The impairment after amygdala lesions, however, not being attributable to a recognition disorder, appears to reflect instead a disorder of memory consolidation. The results thus point to object recognition and object-reward association as processes that depend in part on the sequential participation of area TE and the amygdala.

LOCALIZATION OF LESION IN A NOTED CASE OF CHRONIC ANTIERGRADE AMNÉSIA. Larry H. Squire and Robert V. Moore. Departments of Psychiatry and Neurosciences, UCSF, La Jolla, CA 92039.

In man, chronic anterograde amnesia can result from dysfunction of 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 are the most 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 deficit other than memory loss. The deficit 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 mamillary bodies may result in amnesia in some cases. The results from this patient emphasize the importance of the dorsal thalamus in memory functions.
A PROPOSED EXPERIMENT FOR THE INVESTIGATION OF THE "MEMORY OF SPEELD". George Vrouillis, Dept. Psychophysiology, TRIMS, Med. Center, Houston, TX 77030.

An experimental task 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 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, and 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 reëhearsal phase, which, as the rehearsal 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 in 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 EEG 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, whereas 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.

ENCODING DEFICITS IN ANTEROGRADE AMNESIA. C. Douglas Wetzel and Larry R. Squire (SPON: W.T. Schlapfer).

Departments of Neurosciences and Psychiatry, UCSF, School of Medicine, La Jolla, CA 92039.

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 shared 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 led 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.

VARIATIONS ON A THEME BY LASHEFY: LESION EXPERIMENTS ON THE NEURAL MODEL OF ANDERSON ET AL. Charles C. Woolf (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 neural elements participate in memory. Here I report simulated lesion experiments on neural elements in the Anderson et al model that are similar in spirit to Lashefy'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 syntaptically 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 them. The output of the model is a vector product of the activity in the input and output neurons. This mechanism of association is closely related to that originally proposed 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 produce axiomatic examples of Lashefy's concepts of mass activity. 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 NIH Grant MH-05266).
MONOAMINERGIC SYSTEMS

In the past, we have demonstrated that damage to the median nucleus of the raphe (MR) results in a number of behavioral deficits which arc as a whole to following hippocampal damage. For example, we have reported that MR lesions retard extinction of a straight alley task (Neurosci. Abstr.2, 1976) decrease distractibility in the shuttle box test, and impair reversal of single alternation response (Neurosci. Abstr.3, 1977). To further investigate the nature of this behavior, control and lesioned rats were run through two reinforced trials in a T-maze. During the first trial, one arm of the maze was blocked so that no entrance into that side. During the second trial, that side was left open, and before a third reach was made, 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 hippocampal, fail to perseverate forced chooses.

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. HR animals were found to acquire the initial response as readily as controls, but were clearly impaired on the reversal. This result was obtained with animals trained to a criterion and in subjects given extensive over-training. Acquisition and reversal of a brightness discriminating task was also discussed.

In another test, the effects of median raphe damage on a conditioned emotional response (CER) were assessed. Control and HR rats were trained to a CER with a single session, 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 significant larger suppression of drinking than did HR animals. Impaired acquisition of the conditioned response was obtained for both lesion types.

Finally, PFCs have recently been reported to eliminate latent inhibition in a shuttle box (Solomon et al., P.A.1978). We now report the similar observation that pre-exposure to the conditioned stimulus impairs shuttle box acquisition in control but not HR subjects. Since hippocampal, 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. Berkant and George K. Aghajanian, Department ofPsychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Several lines of evidence indicate that the serotonergic (5-HT) cells of the dorsal raphé may be involved in the regulation of several monoaminergic inputs to the brain. Drug treatments which impair adrenergic tone reduce 5-HT cell activity. For example, administration of either (1) clonidine (0.1 µg/kg, i.v.), (2) guanethidine (30 mg/kg, i.v.), an increase in impulse flow, (Svensson et al., Brain Res., 92:291, 1975) or (2) reserpine (0.5 mg/kg, i.v.), which impairs noradrenergic neurotransmission, suppresses the firing rate of 5-HT cells in the dorsal raphé.

An α-adrenergic antagonist is implicated in the suppression produced since the central effect of α-adrenoceptor blockade in the dorsal raphé can be abrogated by a high dose of clonidine (200 µg/kg, i.v.), which acts as a central post- synaptic α-adrenergic antagonist, whereas a low dose of clonidine (20 µg/kg, i.v.), which acts as a central pre-synaptic α-adrenergic antagonist, has no effect or increases the firing rate of 5-HT cells in the dorsal raphé.

In conclusion, we see sex differences in the spontaneous release of NE and DA, the latter of which is consistent with a variety of determinants, including the posterior pituitary. NE release can be modulated by changes in ions and drugs. Therefore, we propose the perfusion system of brain fragments as a valid in vitro technique for studying endogenous neurotransmitter release. Supported in part by RIAS Grant (NSF SER 76-18255).

828 EFFECT OF SYSTEMIC APOMORPHINE ON FIRING RATES OF Dopamine CELLS IN VITRO WITH STRIATAL KAINIC ACID LESIONS. N.O. Barlow, J.B. Walters, E.K. Silbergeld, N. Eng*, S. DeSantistimon, and J.M. Labosky, Experimental Therapeutics Branch, NINDS, Bethesda Md. 20014.

Systemic apomorphine (APO) causes a rapid decrease in firing rate of nigral dopaminergic (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. In this study, we examine the latter. In the systemic feedback from the striatum, we recorded firing rates of DA cells in rats with striatal kainic acid (KA) lesions, since KA is thought to have a direct effect on DA cell bodies and DA output. Rats were unilaterally injected with 2 µg KA in 1 µl NaH2PO4 buffer at stereotaxic coordinates (Koentig & Klippel) A 7.3, L 2.6, and 5.7 mm from the skull surface. After injection, the striatal DA content and DOPA accumulation after DOPA decarboxylation were 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.

In the cerebellar cortex, units recorded 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 2 days after KA injection. APO was given at 2 min intervals increasing amounting which doubled the previous cumulative dose; cumulative doses ranged from 32 to 64 µg/kg (0.01 to 0.02 mg/kg). While the last half of 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 control rats was APO as effective as in KA-lesioned rats. While there was no difference in the interaction of 5-HT inhibition between control and KA-lesioned rats, there was a significant difference in the average dose which produced 50% inhibition. This may agree with other workers (Bla[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 decrease in the firing rate induced by 5-hydroxytryptamine is not reduced in KA-lesioned rats.

While this study does not rule out the possibility that loss of striatal input can contribute to the change in firing rate, the decrease seen during recovery of firing or after chronic APO, it supports the idea that the acute effect of APO on DA cell firing is predominantly a direct effect on the DA cell.

Spatial preference, operationally defined as turning preference in a T-maze choice test apparatus, was determined for 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 30 trials (r = 0.61, Student's t = 2.67, df = 14. p < .001). One month after the completion of the ten days of behavioral testing, the rats were decapitated and their brains removed. The striatum, left and right hippocampus were dissected and separately assayed for dopamine in the case of the striatum and the accumbens/tuberce, and for norepinephrine in the case of the hippocampus. The average concentrations of dopamine (µg/g + SEM) were: left accumbens/tuberce 4.51 ± 0.21, right accumbens/tuberce 4.47 ± 0.14; left striatum 8.35 ± 0.40, right striatum 7.95 ± 0.49. The average concentrations of norepinephrine (µg/g + 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 was 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, respectively. These findings support a role for dopaminergic asymmetries in spatial preference and focus attention on mesolimbic dopaminergic asymmetries as a possible substrate of spatial preference in intact animals.


The activity of serotonin: N-acetyltransferase (NAT) has a circadian rhythm in the rat pineal gland that peaks 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 sympathetic nerves innervating the pineal from the superior cervical ganglion (SCG). However, attempts to measure an increase in electrical activity of these nerves failed to demonstrate a daily rhythm in NAT activity. The magnitude of the increase in NAT activity makes the pineal gland an appropriate system for studying the neural 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.

Albinos rats were anesthetized and the cervical sympathetic trunks were stimulated bilaterally at 10 Hz, using current intensities of amperes that were too small to produce maximal exophthalmes 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 9 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. These experiments demonstrate that increases in NAT activity can be 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.)


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 1 ml 18 second schedule of 45 minutes per session, which generated low rates of responding. After 76 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 reinforcers earned. A second series of dose-response measurements showed a reduced drug effect, and a third administration of the 10 mg/kg dose 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 of MP. Animals receiving MP twice weekly developed partial tolerance to the rate-increasing effects after 23 administrations. Animals receiving MP post-session did not develop tolerance. After 22 doses 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 each training session. 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 #3419.)


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) has been described which saturates at approximately 0.4μM l-norepinephrine (1-NE). In view of the large (>1.0μM) concentrations of 1-NE one would expect to find in the adrenergic system, 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 1.0μM. We have reported (Fed. Proc. 36:381, 1977) that this second system contrasts with uptake, in that it is not blocked by 10μM cocaine; it shows extremely rapid initial rates of accumulation; and it is markedly inhibited by the removal of calcium from the incubation medium. We have named this second system "caine resistant accumulation" (CRA).

We report here that when cortical tissue is incubated for one minute at 31°C in the presence of 1.0μM H-1-NE, if, instead of 10μM cocaine, either 10μM desmethylimipramine or a sodium-free choline chloride medium, or both, are used to block uptake, CRA does not appear to be altered in either amount or calcium sensitivity. We have also examined this system in brain slices, a crude synaptic preparation, from the pons, substantia nigra, and hippocampus. The purified synaptosomes showed a 50% increase in the amount of calcium-sensitive H-1-NE accumulation compared with brain slices, and the decrease in this effect with increasing concentrations of calcium, suggesting that CRA may, like uptake, be occurring in nerve endings. As with uptake, CRA was blocked by millimolar diphosphonol and the regional distribution appears to follow the distribution of NE nerve terminals in the brain. In contrast with uptake, CRA did not appear to be stereo-selective with regard to 1-NE accumulation, but it was suppressed by both 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.
384 EFFECTS OF CHRONIC MONOAMINE OXIDASE INHIBITORS ON CENTRAL NERVOUS SYSTEMS. Iain C. Campbell*, Dorothy G. Gallagher, Dennis L. Murphy and John F. Tallman. National Institute of Mental Health, Bethesda, MD 20892.  

The effects of the monoamine oxidase inhibitors (MAO) were examined in animal models 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 2:1 increase in the free number of adrenergic receptors as measured by the binding of [3H]-dihydroxyphenylal with no change in the affinity of the receptors. This effect persisted for at least 6 weeks following the last 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 correlated with the differential effects of the inhibitors on norepinephrine (NE) levels. Consistent with desensitization observed in β-adrenergic receptor binding studies, electrophysiologic effects of clorgyline or pargyline are also disrupted by chronic pretreatment with MAOIs. Twenty-four hours following the last daily dose of the inhibitor, animals were anesthetized with chloral hydrate and prepared for additional recording of the NE-containing cell bodies in the nucleus locus coeruleus (LC). Minimal to non-existent spontaneous activity of the LC neurons was observed 28 days following the chronic treatment with MAOIs in contrast to control rats (given saline injections) whose LCs displayed the spontaneous firing patterns and rates typical for this region. However following the administration of the α-adrenergic antagonist, piperazine, in chronically treated rats, a return of spontaneous activity in LC cells was observed. Electrophysiologically observed effects of clorgyline or pargyline are currently being investigated in a predominantly β-adrenergic post-synaptic region. This data suggests that continuous occupancy of NE receptors by NE in chronic MAO-treated animals results in the inhibition of NE cell firing and desensitization of NE receptors.


The posterior salivary gland (PSG) of the octopus vulgaris has been paid attention to because many workers due to its high content of monoamines including octopamine and the existence of chromaffin cells. Natan(1971) classified two types of epithelium: type A and B and identified two types 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.

Monamines-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. Spectrophotometric analyses revealed the existence of dopamine-like spectral pattern (excitation max. 410 nm, emission max. 480 nm and after exposure to HCl 400 nm and excitation max. shifted to 380 nm which persisted even after additional minutes exposure to HCl) in the former and serotonin-like spectral pattern (excitation max. 410 nm, emission max. 510 nm) in the latter structures.

Monoamine-containing (MA) cell granules in the epithelial layer were found to be larger and was partly stained by Tramnini-S silver stain for EM embedded sections. MA cell granules were PAS negative in contrast to surrounding most exocrine cells which stained in variable densities for PAS. 

After the observation of glyoxylic acid induced fluorescence preparation, the same specimens were processed for electron microscopy. It was confirmed that containing dense core granules(0.5 to 3 µm in diameter) were embedded in the amorphous peripheral zones which were divided into many compartments by the membranes. MA cells contained both apleuric and peripheral granules and the nucleus were located in the basal portion surrounded by well developed endoplasmic reticulum and Golgi apparatus. The cell membrane permanently preserved to preserve the cell better than glutaraldehyde or formaldehyde.

Most parts of the MA cell surface are covered by the sheath of surrounding cells occasionally seen varicosities were seen to synapse on MA cells.

The present result showed that MA cells compose neurosecretory complex of the PSG suggesting paraneuronic nature of the cell. It is not clear whether MA cells work through endocrine, exocrine or paracrine mechanism.


The activities of tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and dopamine beta hydroxylase (DBH) were measured in the superior cervical ganglion (SCG) of the rat and the rat's thoracic sympathetic trunk in vivo. Continuous stimulation at 10 Hz for 60 min produced a significant increase in enzyme activity and TH levels (p < 0.025). Dopamine levels increased as a result of continual stimulation (60 min) only in the cervical sympathetic trunk in vivo. Continuous stimulation at 10 Hz for 60 min produced a significant increase in TH activity and DDC activity with little change in DBH activity. These results demonstrate that direct stimulation of the preganglionic nerves innervating the superior cervical ganglion produces a significant increase in TH and DDC activities with little change in DDC activity and suggest that the increase in TH and DDC 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 the nicotinic receptors for the octopamine, largely block the increase in TH activity produced by preganglionic nerve stimulation (Chalazonitis and Zigmond, Proc. Soc. Neurosci., 1977). Muscarinic 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 non-cholinergic inputs to nerve fibers. The latter findings were interpreted to suggest that the increase in TH activity produced by preganglionic stimulation may involve the function of non-cholinergic inputs to nerve fibers. Further experiments showed that the increase in TH activity produced by preganglionic stimulation was not blocked by any of the cholinergic antagonists tested. These findings suggest that the increase in TH activity produced by preganglionic stimulation may be mediated by a conformational shift in the state of the dopamine receptor.


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.p.) but not by bromocriptine (200 µg/kg i.p.), and were mimicked by the non-hallucinogenic congener of LSD. These and other electrophysiological data are supportive of the hypothesis, derived from behavioral and neurochemical experiments, that LSD acts as a dopamine agonist in the CNS. In addition, biochemical experiments measuring dopamine specific receptor binding or synthesis of dopamine sensitive cAMP indicate that LSD acts as a dopamine agonist. The present electrophysiological experiments support this 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 75% of the original baseline. A second injection of this dose of LSD further increased the discharge rate, to a point 5% above the original baseline. A similar pattern was observed when this dose of LSD was administered to rats whose nigral cell discharge had been depressed by apomorphine pretreatment (50 or 100 µg/kg i.v.). Bromocriptine, which shares LSD's dopamine agonist, but not antagonist, properties also produced similar effects. Finally, pretreatment with LSD (200 µg/kg i.v.) produced a 25% increase in the dose of d-amphetamine required to produce a 50% decrease in nigral discharge of those rats that were pretreated with LSD. These findings are consistent with 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 pretreatment with dopamine agonists may be mediated by a conformational shift in the state of the dopamine receptor. (Supported by NIMH grant MH-23343).

The prefrontal cortex, dorsal to the rhinal sulcus of the rat has been related to extramartial 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 afferents from the sulcal cortex (Clavier & Corcoran, 1976), no data exist as to the possible contribution of afferents to this region.

As a preliminary attempt 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 ul 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. Histological reaction was with 3,3' diaminobenzidine or benzidine 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 findings (Lindwall 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. Lindwall's group reported A10 cells from the level of the posterior mamillo-nuclear 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 Fitchier, 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 serotonin innervation described by Thomas et al., 1978.

Thalamic inputs to the sulcal cortex included a projection from the mediadorsal 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 neuroanatomical techniques.

Ongoing studies into the possible role of each of the sulcal afferents described herein are discussed.

(Supported by NIH Grant I RO1 MH 30296-01).


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 simultaneous histochemical localization 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 histochemical localization of CA and LHRH (McNeill et al., 1970: 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. Perfusion fixations used were 38 (T. M. Nett) and 43058 (L. A. Sternberger).

Staining in the ME was similar with both antisera, and additional carriers were used to improve specificity of the LHRH reaction product. Occasional deposits of LHRH reaction product were found in the CA-containing zones. Immunoreactive CA neurons were observed in the tuberoinfundibular nucleus, and, unlike the results obtained in freeze-dried tissue, were 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 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.


Endogenous levels of serotonin were measured in several hypothalamic nuclei within the adult male Sprague-Dawley rat using the radioenzymatic assay developed by Saavedra in conjunction with a modification of the Farkovits 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 suprachiasmaticus) 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 histochemical 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) limited, 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 temperatures during removal of the punched out areas. In addition, a reliable staining procedure for 500 of thick unfixed brain tissue has been developed using a modification of the cresyl violet stain (4). Staining is done directly on the slide with cytoarchitectural resolution equal to 50 sections stained with cresyl violet.


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 (SN) may be terminated due to activation of the midbrain DA containing cell groups (A9-A10) or their efferent projections. Several questions still remain regarding the role of the DA systems in the ICSS. First of all, despite marked anatomical differences among the nigrostriatal, mesolimbic and mesocortical DA systems, it is not known whether all DA systems participate in ICSS. Second, the induction 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 were employed to verify electrode placements.

ICSS thresholds decreased and response rates increased as electrodes were lowered from the A10 cell group designated by Lindwall and Bjorklund. Electrodes lowered through A10 into the interpeduncular nucleus ceased to support ICSS. The rostro-medial portion of the A9 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 hypothalamic sites. 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 interpret the results in 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 role that the DA cell group supports ICSS within 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 thresholds, 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 NONOMINE OXIDASE IN CULTURED CELLS WITH A AND B ACTIVITIES. Morris R. Castro Contre, Morris Hawkins, Jr.*, and Xandra O. Brekfeld. Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

Two types of nonomine oxidase (NMO, EC 1.4.3.5) activity can be distinguished on the basis of 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 NMO activity in living cells, homogenates and crude mitochondria from two cell lines. Rat hepatoma line MHC_C expresses both A and B types of activity, while human sarcoma line Bu25 has predominantly B type activity. MHC_C 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 B05 and 200 uM activity. Deamination of tryptamine in Bu25 cells; deamination of phenylethylamine is inhibited by low concentrations of deprenyl. A monophasic curve of clorgyline inhibition of tryptamine deamination has been observed.

Pargyline binds specifically and reversibly to nonomine oxidase (Chuang et al., J. Biol. Chem. 249:2381, 1974). We have used [3H]-pargyline to label this enzyme in crude mitochondrial preparations from both cell lines. Following binding of [3H]-pargyline, mitochondrial proteins are solubilized in sodium dodecyl sulfate (SDS) and separated by SDS-polyacrylamide gel electrophoresis and isoelectric focusing (Ames and Nkido, Biochem. 15:616, 1976). The location of labelled proteins is determined by autoradiography via [3H]PIL-1. Several labelled proteins can be identified and their labelling is blocked by 0.4 uM clorgyline. The migration pattern of these proteins is similar in MHC_C and Bu25 and with A and B activity, and only B activity. Thus the presence of A and B types of NMO activity does not appear to correlate with specific protein species.

843 EFFECT OF LOCUS COERULEUS STIMULATION ON REGIONAL BLOOD FLOW IN MICE. Richard L. Delaney, Wickcliffe C. Abraham, Steven F. Zornetzer, and Adrian V. Vane. Department of Pharmacology, University of Calif. College of Medicine, Gainesville, Fla., 32610.

The effects of unilateral locus coeruleus (LC) stimulation on regional cerebral blood flow (rCBF) were examined in rats. 5-mg/kg of amphetamine or antipyrine was given to each animal as an index of perfusion. Both anatomical and physiological evidences have suggested that the LC is involved in the regulation of the cerebral blood flow. The experiment was designed to investigate this association of LC with cerebral blood flow in various regions of unanesthetized, 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 0.05 uM solution of tyramine 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 uCi of [3H]deoxyglucose by means of a tubing extension connected to the jugular cannula, and forty minutes later, 1.5 u Ci of [14C]antipyrine. Concurrent with the antipyrene injection, the LC was stimulated at two different threshold currents using biphasic pulses for sec. Those stimulus parameters were effective in altering behavior in another experimental situation. The behavioral change was associated with a significant increase in rCBF. This experiment was repeated with a second set of animals, using the same LC stimulation and antipyrene injection, but after one of the innervation systems was destroyed. The results of this experiment will be discussed in the context of our previous findings on the effective stimulation of various LC afferent systems and the regional cerebral blood flow in the unanesthetized, unrestrained mouse.


The catecholaminergic (CA) innervation of the forebrain of birds was studied in chickens (Gallus domesticus) of 3 days to 1 month age. Some of the animals were killed by decapitation and their brains processed either according to the Falck-Hillarp paraformaldehyde method (Falck et al., Acta. Path. Microbiol. Scand. 49:1) or to the crystalloidal glassy acid-glyoxylic acid procedure of de la Torre and Surgeon (1976). Other birds were anaesthetized and perfused with a solution containing glacial, 1M acetic acid and high concentrations of magnesium and calcium. The brains of these animals were then processed according to the combined formaldehyde-glyoxylic acid method of Loren et al. (1976).

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 ependymal structures. Several of these are the dorsomedial and dorsocaudal anterior thalamic nuclei and the dorsomedial posterior thalamic nucleus. A very large number of CA varicosities is also found within the hypothalamus and the 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, DBH-containing 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, varicose fibers are found in the olfactory tubercle, the nucleus accumbens septi, the lateral septal nucleus and the so-called locus paracolliculi. A stratum of CA varicosities on the basis of the olfactory tubercle and olfactory bulb appears to be distinguished. Several varicosities also occur in the paleostriatum augmentatum, especially within its dorsomedial portion. In contrast, the paleostriatum profundum contains few varicosities. A few scattered, discrete varicosities occur in the mesencephalon where the fibers often lie just beneath the pial surface. In the caudal part of the telencephalon, the number of CA varicosities increases significantly in the nucleus accumbens septi and the hippocampal formation. These varicosities are often lying just beneath the pial surface. In the caudal part of the telencephalon, the number of CA varicosities increases significantly in the nucleus accumbens septi and the hippocampal formation. These varicosities are often lying just beneath the pial surface. In the caudal part of the telencephalon, the number of CA varicosities increases significantly in the nucleus accumbens septi and the hippocampal formation. These varicosities are often lying just beneath the pial surface.
RAPHÉ NUCLEI IN THE RAT: EFFERENT PROJECTIONS TO FOREBRAIN STUDIED USING THE HORSEBEARD PEROXIDASE-BETAGRADE 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 rabbit by the HRP method (0.04–0.3 µl; 30% HRP) to demonstrate efferent neuronal connections. The injections were localized into the raphe nuclei (DR), nucleus centralis superior (CS), nucleus reticularis tegmenti pontis (NRP) and nucleus raphe pontis (NRP).

Studies demonstrate the topographical features of the ascending projections of midbrain raphe nuclei. First, DR has the most extensive projection showing heavy labeling after injections in all areas of the neocortex, mesocortex, 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.

Studies show that the control of the cell body of the raphe neurons is by control of dopaminergic tone. The raphe neurons show the release of DA in a variety of conditions including stress, pain, and reward.

BIOBEHAVIORAL JOURNAL, 1978, J. Comp. Neurol. 180 (3). This study was supported by USPHS Grant NS-12080.

BEHAVIORAL AND ELECTROENCEPHALOGRAPHIC CORRELATES OF LOCUS COERULEUS NEURAL 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. Elizabeth’s Hospital, NIH, Washington, D.C. 20032.

Discharge activity of individual locus coeruleus (LC) neurons was recorded in squirrel monkeys. Anesthesia was induced by an intraperitoneal injection of ketamine (30 mg/kg) and xylazine (5 mg/kg). A pair of stimulating electrodes were used to deliver 0.2 ms duration, 200–400 µA electric shocks to the peduncle of the midbrain. The recording electrodes were placed in the substantia nigra pars reticulata and the pedunculus. The stimulation parameters were adjusted to achieve a 50% increase in the number of action potentials in response to the stimulation.

The results showed that the discharge activity of LC neurons was increased during the post-stimulus period. The increase was observed in all animals and was not due to general anesthesia.

The data suggest that the LC neurons are involved in the modulation of motor activity in response to sensory stimuli.

Septal lesions made in female rats exposed to 100 IU rLH/day from the 3rd day of pseudopregnancy to 18 days post-partum induced similar, correlated changes in female and male rats castrated males 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 the 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 = 10.05, n = 16) or normal males (LQ 0.00, n = 20). Six weeks following the 1Q test the three groups were subdivided and treated with ACUTE-EB or oil and decapitated. The brains were removed, frozen and stored at -5°C prior to dissection and post. Reactive estrogenesis (TH) activity was assayed in the dopamine (DA) rich areas of the forebrain (striatum, STN; nucleus accumbens septi, ACB; and olfactory tubercle, OLT). The TH activity in the SL-EB group was significantly suppressed in both the STN and ACB relative to all other groups. The glutamate decarboxylase (GAD) activity in both the substantia nigra pars compacta and ventral tegmental area (VTR) was significantly increased in the SL-EB group ACUTE-EB relative to all other groups. In summary, the SL-EB group given ACUTE-EB (2) decreased DA content, decreased DA activity (DA 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 content, and 3) increased TH activity, relative to sham animals given ACUTE-EB. Thus, a decrease in DA activity in both males and females is associated with an increased TH activity of the DA cell bodies (indicators of increased TH activity in the DA cell bodies). Since pharmacological and neurochemical data indicate that dopaminergic inhibition on 3 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.)


There is ample evidence that adenylyl 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 cyclic AMP in capillary beds of CNS may be regulated by CNS norenergic neurons. In addition, catecholamines and vasopressin modify CNS capillary function. To determine whether cerebral capillaries contain catecholamine-sensitive cyclic AMP responsive systems, 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 pmol/g protein. Drug treatment with 100 nM 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 ED50 of 3 nM. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (40 nM) increased basal levels of cyclic AMP by 50%, but did not alter epinephrine and synephrine-induced cyclic AMP elevation. Since cyclic AMP levels in cerebral microvessels, but phenylephrine, dopamine, serotonin and histamine did not. Propionitrite completely blocked the relaxation of NE, whereas an equal concentration of phenolamine did not alter its effect. These results indicate that cerebral capillaries contain a cyclic AMP system which is regulated via B-adrenergic receptors and support the contention that cerebral capillary function is under neuroendocrine 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-06333 and MH-13852).


There is evidence in the literature that ephinephine (E) is both localized and synthesized within several regions of the CNS. To determine whether ephinephine neurons were present and whether immunohistochemical techniques were 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) are killed by decapitation and the brains quickly removed onto dry ice. After cooling for 1-1½ hr, they are mounted in a cryostat, sectioned at 15-10, microns. Sections are picked up onto glass slides pre-cleaned in methanol. Slides are then incubated 30 min. with rabbit anti-serum containing antibody to synephrine, washed, fixed briefly in 0.5% w/v parafomaldehyde, washed again, then incubated a further 30 min. with an antibody to rabbit IgG globulin with FITC. These latter antibodies are then incubated in normal rabbit serum at the same dilution as that containing the antibody or with anti-serum pre-saturated with E.

Consistent staining has been found in several regions of the CNS on slides incubated with antisera directed against controls. Among them are several of the hypothalamic nuclei, the nucleus interstitialis of the stria terminals, nucleus septi lateralis, amygdala, hippocampus, and periaqueductal gray. The results (1) tend to confirm the distribution of E as reported in the literature, and 2) do not correspond to the distribution of norepinephrine.


Single unit activity (ups) was recorded from the dorsal raphe nucleus of freely moving cats (n=12) by means of movable 32u or 67a nichrome wires. Virtually all ups were discharged with the spiking pattern that characterizes raphe neurons recorded in vivo and in vitro. Across the sleep-waking cycle, these cells discharged at increasing rate with level of arousal, as defined by both behavioral and electrographic criteria. From a mean baseline firing rate of 2.8 spikes/sec during quiet waking, the mean discharge rate of these cells gradually 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 EOG synchronization) as compared to quiet waking, and then progressive decrease during the early (34%), middle (52%), and late (68%) phases of slow wave sleep. During all phases of slow wave sleep, the mean discharge rate of ups was correlated with a significant decrease in unit activity. Raphe unit activity showed decreases of 62% during REM (likely 60 sec-1) and 100% during REM, as compared to quiet waking. Unit activity reappeared 3.2 sec before the end of REM, with significant increases of 130% during the first sec and first 10 sec, respectively, as compared to quiet waking. These data, in conjunction with a decrease in behavioral arousal, suggest that the role of CNS serotonin may be compensatory to behavioral arousal, and furthermore, that it may play a modulatory, role more than a mediating role in behavioral and physiological processes. (Supported by MMR grant MH-23433).
854 ONTOGENY OF UPTAKE AND RELEASE OF $3^\text{H}$-CATECHOLAMINES IN BRAINS OF RATS NEONATALLY EXPOSED TO LEAD. Kathryn M. Jason (Spon: R. 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 gavage. Striatal and cortical slices were incubated with $10^{-7}$ M $3^\text{H}$-dopamine ($3^\text{H}$-DA) or $3^\text{H}$-norepinephrine ($3^\text{H}$-NE), respectively. Lead treatment had no effect on uptake of $3^\text{H}$-NE in the cortex. However, uptake of $3^\text{H}$-DA in the striatum was altered by lead at both 15 and 35 days. At 15 days, less $3^\text{H}$-DA was present in striatal tissue cores of 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 $3^\text{H}$-DA was present in the high lead group than controls, and even more $3^\text{H}$-DA was present in the cortex, again 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 $3^\text{H}$-NA from the cortex, resulting in decreased release. Possible sites for this alteration were in intracellular binding sites for NA, or may act to replace the calcium necessary for spontaneous release.

(Supported in part by NIMH grants: NS-11752, and NIH grants NS 10777, ES 01247, and ES 01248.)


The nucleus accumbens (N.Ac.) receives dopaminergic projections from the ventral tegmental area (VTA), and has also been implicated in amnulatory activity. Increased amnulatory 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 amnulatory activity. The experiments also investigated the contribution of the suggested GABA-ergic pathway to the GP to amnulatory activity.

The administration of DA (10 and 20 μg) into the N.Ac. increased amnulatory activity in an open field test. The combined administration of DA and carbamazepine (cholinergic agonist, 5 and 10 μg) into the N.Ac. elicited an increase in amnulatory 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) did not attenuate the DA-stimulated increase in amnulatory activity. Amnulatory 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 amnulatory activity. This 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 amnulatory activity. (2) Cholinergic synapses within the N.Ac. appear to modulate the DA-stimulated amnulatory 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. D.L.J. is the recipient of a Medical Research Council Fellowship.)


The 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 un restrained 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, sub-dural 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 antagonistically 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 baseline resting levels of about one to ten Hz., with a latency usually between twenty and fifty msec. in response to gross auditory or visual stimulation; qualitative tactile stimuli evoke similar results. Most LC cells showed multi-modal responsiveness. 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-wake cycles, discharge rates were highest during 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 rates 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 NIIAA Grant GA 03044, and Weizmann Institute, Rehovot, Israel.)
858 IMMUNOHISTOCHEMICAL DEMONSTRATION OF CATECHOL-O-METHYLBTRANSFERASE IN MAMMALIAN BRAIN. Gary P. Kaplan*, Boyd K. Hartman and Cyrus R. Creveling*. Dept. of Psychiatry and Neurology, Northwestern University School of Medicine, St. Louis, MO 63110 and Laboratory of Chemistry, NIMH, Bethesda, MD 20014.

The specific immunofluorescent staining for catechol-O-methyltransferase (COMT) (EC 2.1.1.16) was obtained in rat liver and kidney and in several areas of rat, chinchilla, and bovine brain. Tissue sections were fixed for 70 days in 4% formaldehyde-Vibratome and equilibrated with a 15% sucrose solution. A specific antiserum prepared against rat liver COMT was used on frozen tissue sections followed by incubation with fluororescin isothiocyanate conjugated anti-rabbit globulin. Controls consisted of sections prepared as described above, with normal rabbit serum replacing the specific antiserum and in the absence of the antiserum. These 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.


The intermediate nucleus of the thoracic spiral cord (IML) and the dorsal motor nucleus of vagus (DMV) were examined by immunohistochemical fluorescence microscopy, using antibodies against 5-hydroxytryptamine (5-HT), substance P, enkephalin, somatostatin, and tyrosine hydroxylase. In IML, 5-HT fibers, some of which originate from the lateral and ventromedial hypothalamus, were observed as varicosity-containing fibers. In DMV, various monoaminergic fibers were observed. In IML, immunofluorescence was observed in the intermediate zone, which was the predilection site for IML with extrinsic monoaminergic nerve cells. In DMV, monoaminergic nerve cells were observed as varicosity-containing fibers. In IML, immunofluorescence was observed in the intermediate zone, which was the predilection site for IML with extrinsic monoaminergic nerve cells. In DMV, monoaminergic nerve cells were observed as varicosity-containing fibers. Immunohistochemistry with antibodies against 5-HT, substance P, enkephalin, somatostatin, and tyrosine hydroxylase was performed. In IML, there were prominent varicosities containing 5-HT, substance P, enkephalin, somatostatin, and tyrosine hydroxylase. In DMV, there were prominent varicosities containing 5-HT, substance P, enkephalin, somatostatin, and tyrosine hydroxylase.

860 THE BEHAVIORAL EFFECTS OF AMPHETAMINE ARE CORRELATED WITH THEIR 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 adenylyl cyclase in vitro, and DA receptors 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, there was a 35% increase in striatal CAMP levels. At 10 mg/kg, amphetamine increased the CAMP content of both frontal cortex (39% p<0.01) and striatum (32% p<0.01). Treatment with fluhenazine (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 analysis showed that given in the presence of D1-like dopamine receptors. Treatment with fluhenazine (0.5 mg/kg, s.c.) pretreatment then, a 30-60 min later. Rats given 3 mg/kg amphetamine showed an increase in locomotor behavior while rats given 10 mg/kg showed a decrease in behavior. Behavioral effects were blocked by fluhenazine (0.5 mg/kg, s.c.) pretreatment. Thus, locomotor behavior was correlated with elevated CAMP levels in frontal cortex. While stereotypy was associated with the two behavioral effects. Fluphenazine may involve different brain regions. They further suggest that fluhenazine-sensitive elevations in CAMP may be useful as an index of DA release.


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 varicous processes was therefore studied by taking advantage of the formaldehyde-Vibratome fluorescence method (Hamburger 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 (Gi Spectrum and Southe, 1977). This method successfully demonstrated the fluorescence of both DA cell bodies and their fine varicosis-containing processes in rats pretreated with 1 mg/kg 5-hydroxydopamine (5-HDO). The fine (fluorescent) varicous processes originate from DA cells and were clearly seen in both normal and 5-HDO treated rats (desmethylinproline pre-treatment) material. Comparison of fluorescent varicous and thionin phorons prepared from identical sections disclosed that many fluorescent varicous processes seen in the pars reticulata (SNR) were indeed nigra, as opposed to asynaptic. In the dorsal area of the SN, cells which stained with thionin were densely distributed in the pars compacts (SNc), while SNR cells were rather sparse. The nadded section was used for SNR cells. Using SNR cells, the ventral and lateral varicous processes formed dendritic bundles. When expanded caudally, there was a noticeable increase in SNR cells, SNR-DA cells and dividing into two bilateral, namely mediocr and lateral. At the level of the exit of the oculomotor nerve, a considerable number of non-DA cells were observed among the ventral SN-DA and non-DA cells. At this level, the medial SN-DA cells could not be delineated from A10 DA cells. Those A9 DA cells, however, tended to lie parallel to the lateral border of the pars reticulata (SNR). The fine dendritic varicous processes were seen to run dorso-laterally towards the lateral SNR cells. Some of these processes, then, tended ventrally and were associated with varicose, presumably interneurones, which lie below the A9 DA cells. Perforations through this layer of small neurons, these dendritic processes further extended adjacent to the large deeply-stained (Nissi) non-DA cells present in the ventral ventral level of SNR. The present results thus suggest a close association between the A9 cells and non-DA pars reticulata neurons via the long dendritic varicous processes originating from A9 DA cells in SNR. (Supported by NIMH 25281 and NSF 19388 to A. R.)

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 IPSF. However, this consideration has not been confirmed in electron microscopy.

The SIF of rabbit sympathetic ganglia, thickened neuronal profiles packed with numerous small granular vesicles (65 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 µm² 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 neurone. Occasionally these two neuronal profiles were directly apposed to each other without any membrane specialization. These monoaminergic neuronal profiles survived the preganglionectomy. Five monoaminergic neuronal profiles were followed up to 300 µm in maximum length using 300- and 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 0.3-0.2 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 to series of varicose terminals. No cytoplasmic processes showing features of SIF cell processes were seen in the present materials.

The present results show 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 to be generated for the slow IPSF than the ganglionic SIF cells.


The monoamine precursors L-tyrosine, L-phenylalanine, and L-tryptophan were administered by either oral or subcutaneous routes to young adult mice. The behaviors of the mice were observed up to 72 hours after administration. Each mouse received both the monoamine and a corresponding standard. The mice were then tested in the Lattes test for a 24-hour period. The L-tryptophan induced the most aggressive behavior, followed by L-phenylalanine and L-tyrosine. The chemical characteristics of the monoamines have been shown to play a role in the development of aggressive behavior. The results of this study indicate that the monoamines have a significant effect on the development of aggressive behavior.

Although different facets of the projections of the locus coeruleus (LC) have been studied using autoradiographic, immunohistochemical and immunocytochemical methods, no attempt has been made to characterize the total efferent output of this nucleus. We have placed small injections of 3H-5-HT into the LC in rats and after 4 to 7 days survival the brains and spinal cords were processed using the autoradiographic method. Ascending fibers travel primarily through the noradrenergic midline raphe nuclei and may enter the reticulotegmental nucleus of the pons and periaqueductal gray, where they synapse with neurons in the reticular formation ventral to the vestibular nucleus and turn ventrolaterally along the medial edge of the spinal trigeminal nuclei and medulla. The descending fibers arborize in the ventral 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 (1975). Two notable exceptions are the noradrenergic projections to the suprapontine nuclei of the hypothalamus and the dorsal accessory olive which do not appear to arise from the LC.

(Supported by USPHS grant NS-2751 and American Heart Association grant 77-179)

DEVELOPMENTAL ORGANIZATION OF SEROTONIN-CONTAINING CELL GROUPS WITHIN THE BRAINSTEM RAME OF THE RAT. Pat Levitt* and Robert V. Moore, Dept. Neurosciences, Univ. Calif., San Diego, La Jolla, CA 92037

Recent autoradiographic and horseradish peroxidase studies from our laboratory (Palton and Moore, this volume) have indicated that some cells of the fused midline raphe nuclei in the mesencephalon project ipsilaterally to forebrain structures. Those same nuclei in serotoninous rats. Fluorescent 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 137, 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 (Olsen, I. et al., 1972, 2, 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 dorsal-ventral or mediolateral direction. Cell groups E3, B6, and B9 (dopamine neurons) are 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 rostro-caudal gradient. On postnatal day one (P1), the E7 group is completely fused; B8 is partially fused, while the caudal pontine and medullary cells remain paired. On P5, all the midline groups have fused except B1 and B2, which have undergone a partial fusion. The two caudal groups fuse by P6.

These 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 evidenced by the apparent projections of some of the raphe neurons. Supported by USPHS Grant NS-12080.


Arjimo and Fidzési 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 endogenous aromatic amino acid decarboxylase activity. The present investigations attempted to assess the importance of central serotonin and dopamine containing structures in the control of ventilation. In 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 record changes in respiratory rate and depth. Anesthesia was maintained with 0.7% halothane in oxygen.

The mean dose of 5HTP (3.1-25mg/kg, i.p.) in pargyline pre-treated rats (50mg/kg 60 min before 5HTP) produced a progressive depression of tidal volume and minute ventilation 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 rate in pargyline pretreated animals and a slight decrease in tidal volume. Haloperidol (2mg/kg, i.p.) was able to antagonize the apomorphine-induced increase in respiratory frequency. Mechanical treatment with 5-hydroxytamine to reduce brain dopamine and norepinephrine content produced an increased response to apomorphine when adult.

These results suggest that central serotonergic and dopaminergic neurons may modulate respiratory activity by influencing the respiratory frequency and tidal volume of ventilation.

2-PHENYLETHYLAMINE UPTAKE BY RABBIT RED BLOOD CELLS. Michael F. Masser* and Aron B. Nagar, Dept. of Pharm., 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 phenylethylamine neurohumoral mechanisms (Non-catechol 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 3H-PEA (1x10^-6M-1x10^-7M). After incubation, the RBCs were rapidly separated, digested with 70% perchloric acid, decolorized with 30% H2SO4, and counted. The buffer fraction was also assayed for 3H and 4C. In all the experiments, C14 sucrose was added as a plasma marker; all values were corrected to hemocrit of 50%. Percent uptake of PEA was relatively constant, 58% of the total recovered radioactivity over the range of 10^-9 to 10^-7M (n=86). Lysol occurred at higher concentrations. Uptake was constant at 2, 105 and 30' incubation, showing some degree of saturation. Incubation temperatures (freeze-thaw method) exhibited a concen.RBC/concent. 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% (cmg/cm_0=0.79). Preincubation with 10^-6M (n=30, 30 min.) iodoacetamide or 10^-5M 2,4-Dinitrophenol (n=6, 30 min.) had no effect. In a glucose free (sucrose substituted) medium the cmg/cm_0 was unchanged. Uptake showed no temperature dependence (6°C incubation compared to 37°C). In a similar manner, uptake of 3H-DOPA in the erythrocytes was about 60% (n=10).

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-55563 and by Univ. of Health Sciences/Sch. of Grad. & Postdoc. Studies.

MONOAMINERGIC SYSTEMS
870 NORADRENALINE - ACETYLCHOLINE INTERACTIONS IN BRAIN:
BROVIADIAL FUNCTIONS. Stephen T. Mason and Mars G. Filiger,
Dep't. Psychiatry, Univ. British Columbia, Vancouver, Canada.

It is well established that a noradrenergic-cholinergic inter-
action 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 antagonists,
ascurine 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
muscarin 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 noradrenergic
- - - acetylcholine interaction in the central nervous system may
have a functional role in arousal processes, with catechol-
epinephrine 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 DEACTI-
VATION. Joseph N. Hamsten and Norman Neisler. Dept. Pharm.,

Previous work in our laboratory has demonstrated that various
stresses (decapitation, paralysis, 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 for 15 seconds. Transcorribly for 0.2
seconds. This produces a consistent tonic-clonic seizure lasting
approximately 30 seconds. Following ECS the animals were in-
jected 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 propor-
tion of cyclic AMP-independent protein kinase in the adrenal
medulla, which slightly preceded the maximal increase in cyclic
AMP. It thus appeared 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 hydroxylation with activation of cyclic AMP-
derpendent protein kinase.

This research was supported by USPHS grants NS07927, and
NS09199.

872 THE DEVELOPMENTAL COURSE OF THE RAT BLOOD BRAIN BARI-
ER. To RO 4-4602 Clyde B. Nashura, John Marshall, William C. Curtis and
D.C. In an effort to determine the developmental course of
the blood-brain-barrier to the penetration of a pe-
ripheral DOPA decarboxylase inhibitor, and 10-day
old rats were injected intraperitoneally with eith-
er 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. The brains were analyzed for
the measurement of nanogram quantities of the acidic
metabolites of dopamine, homovanillic acid (HVA) and
dihydroxyphenylacetic 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 N2, methy-
lation in lipopure methanol, derivatization of the acidic
metabolites, and finally the Me/PFP derivative being injected into the aparatus, a Finnigan Model
3000 D Quadrupole GC-Mass Spec employing an 8ft 1/8
inch I. D. 32 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 inhibi-
ting the activity of the enzyme. Additional data
on differential regional permeabilities and other de-
velopmental 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 NBS grant number RR-0816-08.

873 STRAIN DIFFERENCES IN RAT ADRENAL BIOSYNTHETIC ENZYMES AND STRESS
INDUCED INCREASES IN PLASMA CATECHOLAMINES. R. McCarty, G.M.
Glad, V.K. Neisler and J.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 epine-
phrine (EPI) of 5 normotensive rats strains following exposure to 5
min of footshock stress. The most active 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 unstimmed rats of the two strains were also examined.
Activities (moles product/pair/hr) of tyrosine hydroxylase (TH)
and dopamine-D-hydroxylase (DBH) but not phenylethylamine-N-
methyl transferase (PMN) 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.

<table>
<thead>
<tr>
<th>Wistar-Kyoto</th>
<th>Brown-Norway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma CA (gg/m)</td>
<td></td>
</tr>
<tr>
<td>NE basal</td>
<td>438 ± 79</td>
</tr>
<tr>
<td>stressed</td>
<td>2210 ± 230**</td>
</tr>
<tr>
<td>EPI basal</td>
<td>354 ± 46</td>
</tr>
<tr>
<td>stressed</td>
<td>1590 ± 68**</td>
</tr>
</tbody>
</table>

| Paired adrenals | | |
|----------------|----------------|
| TH | 56.4 ± 2.6 | 69.1 ± 5.1* |
| DBH | 429 ± 21 | 580 ± 31** |
| PMN | 35.7 ± 1.9 | 36.4 ± 2.2 |
| NE (μg) | 9.0 ± 0.6 | 16.4 ± 1.3** |
| EPI (μg) | 43.1 ± 2.7 | 43.1 ± 2.7 |

*< 0.05; **< 0.01.

278
MORPHOLOGY


The correlation of the morphological distribution of catecholamine (CA) varicosities and/or peptide-containing terminals (NP-containing perikarya) in the supraoptic (SON) and paraventricular (PVN) nuclei of the rat was examined by a simultaneous histofluorescence-immunocytochemical technique 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 (250-300g) 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 perikarya. Some few NP-containing perikarya in caudal SON appeared contacted, often by varicosities which completely surrounded perikarya. The densest concentrations of CA varicosities in the PVN were located peri-terminally in the parvocellular portion of the nucleus. Periventricularly located immunoreactive perikarya were contacted by CA varicosities. This part of the PVN was less occupied by CA varicosities. Some perikarya in the magnocellular portion of the PVN were also contacted by CA terminals. Many cells which did not stain positively for NP were contacted 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 contribute to the magnocellular perikarya. It may be suggested that CA innervation to PVN neurons may be via an axodendritic mechanism or possibly an axoaxonic type of contact; the latter being supported by the suggestion that a possible subcompartamentalization of the SON for CA-peptide interrelationships may exist. Investigations using site 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 ALTERNATED BY IMMUNOREACTIVITY 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 (DA) neurons projecting from the VTA to the nucleus accumbens contribute to a 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. In some animals, the dose of spiroperidol into the ipsilateral and contralateral prefrontal cortex was increased to 2 µg (in 1 µl). These observations appear to be consistent 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 COERULUS STIMULATION POTENTIATES PURKINJE CELL RESPONSES TO DIFFERENT STIMULATING INPUTS. Barry Mageean and Robert J. Balleine. Bell & Howell, Chicago, IL 60601.

We previously reported that excitatory and inhibitory responses of rat cerebellar Purkinje (P) cells, produced both synthetically and by microinjection of putative amino acid neurotransmitters, were enhanced during neurochemical awakening. Moreover, it was shown that NE, released synthetically during activation of the noradrenergic pathway from the locus coeruleus (LC) to the cerebellum, could modulate responses to isoflurane applications of gamma aminobutyric acid, the basket and stellate cell neurotransmitter. In this study, we examined whether NE could exert similar modulatory effects on P cell responsiveness to different synaptic inputs. Inputs to the Purkinje cell were activated by supra-threshold current injection (3 shoulder pulses, 500 Hz with 1 reaching rate repetitions) of the rat's serotoninergic mossy cell or climbing cell fiber responses recorded extracellularly using glass microelectrodes. Post-stimulatory responses were much the same 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 0.5 msec duration at 100 Hz) that had been shown to be sufficient for directly affecting P cell discharge. In 4 of 7 neurons, sub-threshold LC stimulation enhanced simple spike excitations (from 0.74 to 0.88 spikes/stimulus) elicited via MF input. Post-inhibitory responses, observed after simple excitatory and complex spike excitations of the P cell were also augmented by LC stimulation in 6 of 8 and 6 of 9 neurons respectively. In 5 additional neurons, pure inhibitory responses evoked by cortical stimulation, presumably mediated via NE activation of the LC cerebellar synapses, were greatly enhanced in both duration and magnitude when preceded by LC stimulation at currents which alone elicited no depression of spontaneous discharge. Thus, these data support the notion 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 NS 18677-0014 to DJM)


Adult Sprague-Dawley female (150-175 gms) or male (310-370 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 period of 1 to 4 h. Control animals were injected with a comparable volume of saline injections. In all studies the rats were sacrificed by decapitation between 13.30 and 17.30. The brain of each animal was immediately removed and placed on dry ice. The anterior hypothalamus, arcuate-medial median and anterior talencephalon 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 spectrophotometric procedure. The decline in the content of these amines 1, 2 or 4 hours after the administration of alpha methyl-para-tyrosine (AMPT, methyl enter; 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, while DA turnover in the anterior hypothalamus, arcuate and median eminence was significantly 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). Doses greater than 0.5 mg to 1.0 mg per hour were equally effective. The effects of i.p. injection of 1 mg/kg) administered for 1 or 2 hours was not blocked by atropine sulfate (50 µg) 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 was not blocked by methysergide (50 µg), and the content and turnover of NA and DA in the other two brain areas were not affected by physostigmine treatment or by mecamylamine. Anatomically, 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. Anatomically, 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. Anatomically, the anterior hypothalamus was increased in the telencephalon of female rats after 4 hours of stimulation with physostigmine (1.5 mg/kg), a dosage which was frequently fatal to the rats of the control group. The term "stress" is used here to suggest that the activity of noradrenergic neurons with terminals in the anterior hypothalamus are particularly sensitive to cholinergic input. (Supported by NSF Grant #CFX 76-01876 and Research Scientist Development Award # K2 5 K02 MH00208-01)

The functional activity of catecholamine (CA) neurons in the central nervous system is thought to be a major factor responsible for central regulation of blood pressure. A number of workers have considered the possible relationship between alteration in metabolism of CA which is concentrated on differences in amine content or enzyme activities in brain stem areas known to be associated with blood pressure regulation between strain (Lewin, 1967) 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 (Fed Proc. 76, 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 4 min at 37°C in Krebs-Ringer bicarbonate buffer with either 800 MNE or 800 MDA. The rate of uptake into NE neurons was defined as the difference between NE accumulation with and without 400 nM methyldiphrimine (MDI). Uptake into DA neurons was measured as the difference in DA accumulation in the presence of 400 nM MDI minus the accumulation not blocked by 400 MDA. In the cerebellum where no significant DA accumulation could be measured, the rate of NE uptake was greater in the SHR which was in agreement with the 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 cortical area 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/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 USDA grant ROI-52511.


Genetically obese mice (ob/ob) have significantly higher levels of brain catecholamines than in lean controls (Loden et al., Jr. Res., 131:162, 1977). The effects of pharmacologic manipulation of central CA have not been studied in the ob/ob. We addressed the question of whether the central methyl-pa-tyrosine (o-MPT) to ob/ob and lean littermate controls. Hypothalamic (HT) and telencephalic (TEL) NE and dopamine (DA) levels were studied in the ob/ob after drug treatment. NE levels were unchanged in the ob/ob hypertensive mice. Persistent NE excess was newly synthesized NE, the tyrosine hydroxylase inhibitor o-MPT (300 mg/kg, 4 hours prior to sacrifice) reduced NE levels in the HT and TEL areas of ob/ob, NE levels remained significantly higher in the ob/ob have been included in the RES treatment. Although significant additional decreases in DA levels were produced in the areas studied (indicating that o-MPT treatment was effective in inhibiting tyrosine hydroxylase), this latter treatment did not decrease brain NE content to control levels. Data for the HT sections are presented in the following table (X, u; ± S.D.):

<table>
<thead>
<tr>
<th>Group</th>
<th>Amine</th>
<th>RES only</th>
<th>RES+o-MPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ob/ob</td>
<td>NE</td>
<td>2.15±.28* (n=6)</td>
<td>1.5±.28*(6)</td>
</tr>
<tr>
<td></td>
<td>DA</td>
<td>1.39±.41 (10)</td>
<td>0.7±.23 (10)</td>
</tr>
<tr>
<td>lean</td>
<td>NE</td>
<td>6.4±.04 (6)</td>
<td>0.8±.02 (6)</td>
</tr>
<tr>
<td></td>
<td>DA</td>
<td>6.1±.09 (10)</td>
<td>0.7±.05 (10)</td>
</tr>
</tbody>
</table>

*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 ob/ob there is a reserve resistant NE pool. This pool may be the result of the genetic selection for binding of NE in the ob/ob. The resulting increase provides additional evidence for the presence of an altered central CA function in the ob/ob. Such an abnormal CA function might contribute either directly or indirectly to the abnormal characteristics of the ob/ob such as impaired thermoregulation, overeating, and altered pituitary function. We are currently studying the relationship of the central CSs to several of these abnormalities. (Supported by NIH grant 1RO3NS01679 and RR-05366).


Causal effect relationships between catecholamines and essential hypertension remain tenuous. Large amounts of norepinephrine (NE) or epinephrine (E) released from a tumor are thought to result in hypertension due to phenoxybenzamine. 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 dog. 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; and aldosterone 94 ± 6 pg/ml; plasma renin activity (PRA) 2.0 ± 0.1; plasma sodium (Na+) 145 ± 1.3 meq/l; plasma potassium (K+) 4.6 ± 0.8 meq/l.

Of six dogs submitted to unilateral nephrectomy, 2 served as controls in which the Goldblatt plaque was unabated; the remaining 4 developed chronic hypertension within 3 days after blood supply to the remaining kidney was reduced. The chronic Map was accompanied by a chronic, statistically significant increase in NE (p<.05). PRA and aldosterone rose sharply immediately after application of the clamp. Both renin and aldosterone levels decreased over the next two weeks. Pulse rate after an initial drop, rose over a two week period to stabilize at 188 ± 1 (p<.01). Chronic E levels did not change significantly 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


An antibody to tyrosine hydroxylase isolated from a human pheochromocytoma is being used with the peroxidase-anti-peroxidase immunohistochemical technique to map distribution of similar 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. In addition to substantia nigra, other catecholaminergic fibers further increase in density in prefrontal regions identified. 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 tracks. 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 noradrenergic and adrenergic nuclear groups.

The dimensions and location densities of the stained structures are being determined for rhesus monkey using serial sections cut in three planes. These data together with similar data obtained in man are crucial to the understanding of neurologic disease. A survey of the neuropepatoches of the variety of animal is being initiated. The sympathetic ganglia in familial dysautonomia have approximately 90% reduction in neuron population in some cases while normal TH activity per g well stained with light microscopic methods. Serial sectioning of the ganglia indicates an abnormally high proportion of TH rich neurons which stain more intensely than in control controls. Different survival strategies for different neuron population or functional hypertrophy of persistent neurons may occur. Increase in neuron size supports the latter possibility.
ANTAGONISM BY UPTAKE INHIBITORS OF α-METHYL-D-TYROSINE DEPLETION OF SPINPHINE, NORSPINPHINE AND DOPAMINE BUT NOT THERMOSERUM IN RAT BRAIN. Kenneth W. Perry* and Ray W. Fuller (3. Lilly Research Laboratories, EI Lilly and Company, Indianapolis, IN 46206.

Epinephrine (E), norpinephrine (NE) and dopamine (DA) were measured in rat brain regions by a high performance liquid chromatography method with electrochemical detection [Biochem. Pharmacol. 26, 2087 (1977)]. E was detected in hypothalamus, midbrain and brain stem but not in other regions. All three were present in cerebral cortex. The catecholamines (CA) were depleted by α-methyl-D-tyrosine (100 mg/kg, s.c.) at 6 hrs after injection. DA depletion was greater in the cerebral hemispheres (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-D-tyrosine (αM) injection was antagonized by pretreatment with various CA uptake inhibitors but was not completely prevented. Administration of an uptake inhibitor 6 hrs after αM injection, a time when the catecholamines were already depleted, partially reversed the depletion. However, serotonin depletion by αM was not altered by pretreatment with fluoxetine, an inhibitor of uptake into serotonin neurons. Apparently part of the serotonin (5HT) depletion after αM injection is due to the action of amine products (α-methyl-D-tyramine and metanephrine) that enter the neuron via the monoamine transporter and whose presence at the nerve terminals is maintained by the uptake pumps. Since metanephrine (5HT) is localized primarily in NE neurons and α-methyl-D-tyramine (5HT) in DA neurons [Dorris et al., Exp. Neurol. 179, 10 (1977)], a NE uptake inhibitor would be expected to decrease MA concentration in rat brain after αM injection whereas a DA uptake inhibitor would be expected to decrease MA concentration. MA & MTA were measured by high pressure liquid chromatography with fluorometric detection [Perry & Fuller, Science for Nervous Transmitter Abstracts A711, p. 216 (1977)] by 6 hrs after administration of αM alone or in combination with metanephrine, a CA uptake inhibitor. MA increased but NE concentration in brain was depleted, indicating that it inhibited uptake into NE neurons more than into DA neurons. This finding agrees with the order of selectivity for MA uptake inhibition of the CA uptake inhibitors, NE=NE>DA, which was NE>NE>DA. Other uptake inhibitors studied and their order of selectivity were: desipramine, NE>NE>DA; imipramine, FNE=NE; norpropyline, NE>NE>DA, and amipropyline, FNE>NE. These studies show that αM is a useful tool for evaluating the selectivity of CA uptake inhibitors in vivo.

THE EFFECTS OF BIOGENIC AMINES ON THE OCCIPITAL (VISUAL) CORTEX OF THE CAT. G. B. Heimer and P. Carrow, Research in Neurosciences Neurobiology, Université de Montréal, Montréal, Québec, Canada.

Several evidences in favor of specific afferent pathways containing the biogenic amines dopamine (DA), norepinephrine (NE) and serotonin (5HT) have been reported in the mammalian CNS. In an attempt to determine their functional role in cerebral cortex we examined the reactions of NE, DA, NE and serotonin (5HT) of the occipital cortex of the cat ("enchephalic isola" preparation). We also compared the effects of these biogenic amines to their transmitters and metabolites (DA, NE, serotonin, acetylcholine (ACH) and γ-aminobutyric acid (GABA)). The amines DA, NE and 5HT were found to inhibit the visually-evoked activity of a majority of cells sampled, obtained by extracellular electrode, after injection currents of 50-100 nA during 20 to 30 s usually of prolonged duration, lasting 4-6 min and often associated with some hyperpolarization of the transmitters' cells. The ACh inhibited and hyperpolarized all the tested neurons. This effect (ejection currents of 5-30 nA) was rapid in onset and termination lasting only as long as GA was ejected. The neurotransmitter ACh (50-60 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 ACh was terminated, the neuronal recovery time being shorter than 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 neuronal activity of afferents of visual cells may be inhibited by DA, NE and 5HT and that this inhibitory response differs markedly from that induced on the same cells by GABA. The long duration of the effects of these amines may be compared to the visual evoked activity and on the ACh-induced excitation tend to rule out a simple balance between excitations and inhibitions to the reduction of the visually-evoked response of the sensitivity towards ACh. Although the molecular mechanisms underlying biogenic amine and ACh interactions are currently in debate, it is the hypothesis that the latter may act upon mechanisms closely related to the direct post-synaptic actions of these putative neuromodulators. It should be also re- cognized that presynaptic norepinephrine receptors have been postulated to regulate the release of CA and NE (Reader et al., Brain Res., 111 (1976) 95). Thus different levels of interaction may coexist in cerebral cortex with regard to the effects of biogenic amines and ACh on overall neuronal excitation.


Long-term treatment of rats with haloperidol produces an increased sensitivity to the locomotor and stereotaxic effects of apomorphine (AP) which is also accompanied by an increased [3H]spiroperidol binding in the striatum. The purpose of the present study was to assess the effects of lithium and phosphate on this supersensitivity as measured both behaviorally and by striatal dopamine receptor binding. Rats were treated for 21 days with either haloperidol (3 mg/kg) or lithium (350 mg/kg) or the combination of both for 3 hours after injection. Seven days after discontinuation of all treatments, some animals from each group were treated with AP (0.5 mg/kg) induced locomotor activity and stereotyped behavior. The other animals were sacrificed for assay of striatal dopamine receptor binding. Freshly dissected caudates were homogenized and washed in hypotonic buffer. The homogenates were then incubated at 37°C with tritiated spiroperidol and either (+) or (-) butacemol. AP-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 [3H]spiroperidol was increased in caudates of haloperidol treated rats but not in rats that had been pre-treated with both lithium and haloperidol. Lithium treatment alone did not affect behavioral sensitivity to AP or striatal [3H]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 AP (0.05 mg/kg) to depress locomotor behavior. In addition, supersensitivity to amphetamine following depletion of catecholamines was not prevented by lithium and this supersensitivity was accompa- nanted by a reduction in the sensitivity to lithium treatment. These findings suggest that lithium's ability to prevent recurrent mide-depressive episodes may be related, at least in part, to its ability to stabilize dopaminergic receptor sensitivity. Work is presently in progress to ascertain whether chronic lithium also interferes with the development of catechol- amnergic subsensitivity, as well as supersensitivity of other neurotransmitter systems.


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 neurons in the isthmus contain neurons with identified neurotransmitters. These include the nucleus locus coeruleus (LC), nucleus raphe dorsalis (RD), nucleus raphe centralis (CR), and nucleus raphe dorsalis (RD). A Golgi analysis of the organization of these neurons, 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 raphé 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 signi- ficant 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 neu- rons tend to located near the midline and have a significant portion of their dendrites extending to the dorsal and ventral axis of the nucleus. Smaller neurons, tending to be locat- ed 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 with bi- and tri-locular lumina. However, the DTN has been postulated to regulate the release of DA and NE (Reader et al., Brain Res., 111 (1976) 95). Thus different levels of interac tion may coexist in cerebral cortex with regard to the effects of biogenic amines and ACh on overall neuronal excitability.

(Supported by the MRC, the CRSF and CAFIR)

Previous work in our laboratory has suggested that the response of brain noradrenergic (NE) neurons to severe stress is altered by age or stress. NE depletion 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 fell immediately after stress were reduced to 61.5 ± 3.2% of control in older rats but to only 79.9 ± 2.3% of control in younger rats (p<.01). Like-wise, telencephalic NE concentrations fell 46.5% 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 closely approximates the exposure required to obtain 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 foot shock 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 foot shock 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 foot shock were 85.7 ± 2.7% and 99.6 ± 4.1% of control, respecti- tively. In older and younger rats (p<.05). We also compared the resting levels of NE in the hypothalami and telencephalons of noradrenergic rats to determine whether the hypothalamic NE level did not differ in the two groups but telencephalic NE concentra- tion 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. Furthermore, our observation of elevated resting cortical NE levels in older animals, despite their greater susceptibility to depletion, suggests the possibility that the recently relocated NE pool of older animals may be increased in size in order to compensate for its increased liability. Finally, the absence of elevated resting levels of NE in the hypothalami of older rats suggests that these differences are related to age-related functional changes of NE utilization than do cortical NE neurons.


The locus coeruleus (LC) has been described as receiving a noradrenergic (NA) innervation from the caudally situated A1, A2, and A6 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 neurochemical specificity. A specific mapping technique (in press) that is specific for dopamine-β-hydroxylase (DBH) containing neurons, has recently been developed, based upon the principle of specific binding and retrograde flow of antibody to DBH (ADDH) by noradrenergic neurons. This study reports the application of this highly sensitive and specific technique to the activation of the origin of adrenergic fibers terminating within the LC.

Eight adult male rats received stereotaxic injections of ADDH into the right caudal LC via a glass micropipette in volumes of 0.1 - 0.2 ul 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 ADDH. Proper injection placement was verified in thionin-stained sections.

Following unilateral ADDH was visualized within cell bodies of the contralateral LC, and ipsilateral A2, A6 groups. Substantial numbers of cells were labelled, bilateraly with correlative staining seen in cell bodies visualized by this technique and none seen in the brains of PIS injected subjects. Since epinephrine releasing neurones are situated within the A1, A2, and A6 cell groups, we have been reported to project to the LC, it is not known whether A2, A6 or A1 projections are labelled by ADDH. A1 and A2 cell groups were present in the contralateral LC and have been observed to be much greater than those observed in the ipsilateral LC. Caudal to the LC, fibers were observed in and superficial to the LC, and were the noradrenergic bundle. Ascending noradrenergic fibers of the ipsilateral dorsal bundle could be followed rostrally through the telencephalic structures and confirmed for the LC. This study represents the first indication that the LC nerve projection may be altered during the aging process. The LC innervation may be preserved or even augmented by the A9 and A10 dopamine neurons. This study demonstrates that LC projections may alter during the aging process.

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., Fong & Harvey, J. Comp. Physiol. Psychol. 73, 334, 1973). 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 intracerebroventricular injections of 5,7-dihydroxytryptamine (DHT), 6-hydroxydopamine (HDA), or vehicle (VC). Doses of neurotoxin employed were: 0 (VC), 120, 360, and 1000 nmol each (n=6/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 monoaminergic 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 85% 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 is related to the NE component of the stimulus. Supported by USPHS Grant No. MH 16841 and MH 10461.


The CNS of the marine bivalve mollusc Mytilus edulis is composed of a pair of cerebral, visceral and mantle ganglia, which have been shown to be partially catecholaminergic and dopaminergic neurons. A variety of monoaminergic pharmacological agents have been shown to shift 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 stimulate 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 this 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 molicus edulis. Intracardiac administration of 10 μl of 5, 10, 40 and 80 μg of methionine enkephalin and leucine enkephalin were followed at 30, 60 and 180 min with assays of the CNF 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 produced a 26% increase. No changes were detectable in serotonin levels as a result of these treatments. The effects of naloxone were studied to determine the role of an opiate specific receptor which might be responsible for the effects of the enkephalins. Coadministering 10 to 80 μg of naloxone prevented the change in dopamine concentrations, while no decrease in dopamine levels was obtained in the time course of the experiments. While high affinity opiate receptor binding has yet to be demonstrated in the interpeduncular nuclei, the body suggests the possible presence of an opiate receptor in the CNS of M. edulis. This work was supported in part by grant 1-R01-NS-07641-01 of the N.A.R.C. Program of National Institute of Mental Health.


Independent analyses using catecholamine (CA) fluorescence and neurophysis immunocytochemistry have placed both chemicals in similar hypothalamic loci. Physiological data indicate a possible interaction between CA and magnocellular prolactin. 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 stained for the extracellular signals of sharp fluorescence (McNeil and Sladek, Science 200:72-74, 1978). This technique was applied to the problem of morphological interrelations between these two systems in the monkey hypothalamus. Rhesus monkey hypothalami were freeze-dried, treated with hot formaldehyde vapor, paraffin-embedded and serially sectioned at 14 μm. Pairs of adjacent sections through the paraventricular (PVN) and supraoptic (SON) nuclei were examined for CA fluorescence and either human estrogen-stimulated (ESH) or nicotinic-saline-stimulated (SHS) neurophysis. Immunofluorescent autoradiography to ESH and SON appear to stain oxytocin and vasopressin neurons, respectively. Neuronanatomical maps were prepared of the combined ESH, SON, and CA distribution patterns.

Numerous CA varicosities appeared in juxtaposition to positively-stained ESH and SON perikarya and dendrites in the SOH 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 ESH/SON perikarya was seen in rostral rather than caudal SOH. Rostral PVN contained a high (+) density CA field just medial to the ESH/SON perikarya. A (3+) density field within the area occupied by the ESH/SON perikarya 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 ESH and SON-containing perikaryal fields. In many instances juxtaposition appears to exist between CA varicosities and neurophysis in some hypothalamic nuclei, possibly for functional interactions. It is interesting to speculate that aho-adenrergic interactions might account for the large number of CA varicosities located to the periphery of PVN and SON, but this needs to be explored further.

Supported by (SIS)1642 and (SIS)1009.


Recent anatomical studies in the rat using horseradish peroxidase have shown major afferents to the raphe dorsalis (RD) from the locus coeruleus 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 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 RD. Stimuli consisted of 80-μsec impulses of 0.3-0.5 msec per pulse, 0.2-1.0 mA, 1 or 10 Hz for 15-30 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:

<table>
<thead>
<tr>
<th>Baseline</th>
<th>1 Hz</th>
<th>10 Hz</th>
<th>Dec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 ± 3</td>
<td>1.9 ± 2.4</td>
<td>1.4 ± 2.3</td>
<td>73 ± 27 (n=8)</td>
</tr>
</tbody>
</table>

- A change of 25% or greater from baseline was recorded for cells with firing rate above 1 spike/sec.

Two populations of units shown consistent inhibition after stimulation of the LhB units and cells with a midline baseline rate. For many RD cells the duration of inhibition following individual 1 Hz pulses was about 250 msec. After cessation of 10 Hz stimulation the inhibition lasted for 20 sec or more. RD stimulation also produced substantial inhibition of RD activity with less consistent effects on non-raphe units (n=14).

In addition, LhB stimulation not only inhibited raphe cells but also fast firing neurons in the midline area. (Supported by NSP Grant BNS 77-16512 and funds from Burroughs Wellcome Co.)

We reported (1) the presence of a protein in the 100,000 g supernatant of both CNS and myelenic plexus homogenates with high affinity for serotonin. This protein (SBP) is formed by serotoninergic neurons, binds newly synthesized serotonin, and the binding of serotonin is inhibited by reserpine. We now report serotonin inhibitors of this synthetic derivative of 5-HTP, 5-HTP-7-methyl-5-HTP-7-methylimidazolone. In vitro, 50% inhibition is found with 0.2 and 1.0 μM, respectively.

Studies of interactions of SBP with adrenergic receptors were not affected by these peptides. Uptake of serotonin by synaptosomes was only slightly inhibited (9% at 10 μM). 5-HTP decarboxylase and tryptophan hydroxylase were affected up to 10 μM. The N-acetyl derivative was pot hydrolyzed by homogenates of brain or myelenic plexus. The 1C labelled peptide was taken up at an essentially 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.

In ventricular fluid, SBP caused a biphasic effect depending on dose. The lower dose (10 nanomoles) induces a 40% decrease of serotonin in whole brain, whereas the higher dose (100 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 peritoneal cavity or intraperitoneally caused a 50% increase in pain threshold lasting for several hours, as determined by a pinch-jump test. Since N-acetyl-7-HTP-7-methylimidazolone SBP in vitro SBP effects suggest that SBP is involved in serotonin degradation.

Supported by NIH grant NS-12506.


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 3 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 with a naive group-house mouse of BALB/C strain (the intruder), which was then 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. The second test period aggressors and controls were injected with 400 mg/kg i.p. of α-methyl-p-tyrosine, or saline. All mice were sacrificed 2 hours after injection of 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 the 2-way analysis of variance, an interaction was found to be significantly lower in olfactory tubercle and substantia nigra (sona compacts) 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 tegumentum) 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. These changes are consistent with the increased NE turnover in the substantia nigra and A10 which project to the caudate-putamen and olfactory tubercle, respectively. Thus, isolation-induced aggressive behavior increased NE turnover in the A10 region which may produce decreased DA levels in turner 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.


Considerable evidence has implicated the involvement of cAMP in the induction of tyramine hydroxylase (TH) in neuroblastoma cells. The induction is elicited by analogs of cAMP and compounds which raise intracellular cAMP levels, such as prosta- glandin E1 (PGE1) or the phosphodiesterase (PDE) inhibitor 8-bromo-3-(3-butoxy-4-methoxybenzyl)-2′-imidazolidinone (RO-20-1724). In the presence of the latter two compounds, intracellular cAMP levels are elevated and periaqueductal gray 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 50 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 50-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 cAMP analog alone. TH activity increases by about 20% 48 hours after a 12 hour treatment with 50 mM KCl alone. We conclude that, although changes in levels of cAMP may be involved in the induction of TH in neurnal neumoblastoma 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 NS-17927, NS-19199, and USPHS Research Fellowship Award 1-R01-NS5676.

EFFECTS OF LSD ON BEHAVIOR AND RAPHE UNIT ACTIVITY IN FREELY MOVING CATS. Michael E. Troxler and Barry L. Jacobowitz, Department of Psychology, Princeton University, Princeton NJ 08540.

Single unit activity (n=44) was recorded from the dorsal raphe nucleus of cat brains, 1 minute after insertion of nichrome wires, while simultaneously quantifying LSD-induced behavioral changes (e.g., body flicking and abortive grooming). LSD (25 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. Control cells outside the dorsal raphe nucleus showed no significant change in activity following LSD administration. Although the present data provide some 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 LSDs as to saline during tolerance as compared to the non-tolerant condition.

(Supported by NIMH grant MH-23433.)

It has been established that the synthesis of brain serotonin is dependent on the availability of its precursor tryptophan (tyr) which is, in turn, dependent on the conversion of dietary phenylalanine and tyrosine. These studies were performed in vivo to determine the minimum amount of dietary phenylalanine and tyrosine which are required to achieve maximal synthesis of serotonin. The results indicate that the requirement for dietary phenylalanine and tyrosine is not only dependent on the specific amino acid content of the diet but also on the availability of the two amino acids and their metabolic relationship.


The rate-limiting step for the synthesis of catecholamines is the hydroxylation of L-tyrosine to L-Dopa by tyrosine hydroxylase (TH). The regeneration of L-Dopa from the spent product of the TH enzyme, dopamine, is effective only in the presence of 5HT. Our recent studies have demonstrated that Dopa decarboxylase, which regenerates L-Dopa from dopamine in the brain, is completely inhibited by the Dopa analogs, bromocriptine and lisuride. These results provide a basis for the development of a new therapeutic approach to the treatment of neurodegenerative diseases. The results also emphasize the need for a more sensitive and specific assay for TH.

900 EFFECT OF GABA-MIMETIC AGENTS AND INHIBITORS OF GABA CATABOLISM ON THE ACTIVITY OF THE VENTRAL LATERAL GEMATIC NUCLEUS (VN) IN RATS. R. L. WALTERS, S. N. LAKOSKI, M. K. HICKS, KINCS, NIH, Bethesda, MD. 20014

One model which has been used to explain effects of GABA receptors and GABA precursors on the CNS is the GABAergic hypothesis. This hypothesis states that GABAergic mechanisms are involved in the regulation of brain function. The present study investigated the effects of GABA agonists and GABA antagonists on the activity of the VN in rats. The results indicate that the activity of the VN is affected by GABAergic mechanisms. The study also provides evidence that GABAergic mechanisms play a role in the regulation of brain function.


A dense and uniform serotoninergic (5-HT) input to the amygdala (AMYG) and the ventral lateral gematic nucleus (VLG) from the midepineph, a raphe has been previously described by histofluorescence and horseradish peroxidase methods; physiological and pharmacological studies have supported these findings. The aim of this study was to determine if the serotoninergic pathway to the AMYG and the VLG has been affected by chronic serotoninergic exposure. The data indicate that the serotoninergic pathway to the AMYG and the VLG has been affected by chronic serotoninergic exposure. The data also suggest that the serotoninergic pathway to the AMYG and the VLG has been affected by chronic serotoninergic exposure.

902 MONOAMINERGIC SYSTEMS


It has been established that the synthesis of brain serotonin is dependent on the availability of its precursor tryptophan (tyr) which is, in turn, dependent on the conversion of dietary phenylalanine and tyrosine. These studies were performed in vivo to determine the minimum amount of dietary phenylalanine and tyrosine which are required to achieve maximal synthesis of serotonin. The results indicate that the requirement for dietary phenylalanine and tyrosine is not only dependent on the specific amino acid content of the diet but also on the availability of the two amino acids and their metabolic relationship.


The rate-limiting step for the synthesis of catecholamines is the hydroxylation of L-tyrosine to L-Dopa by tyrosine hydroxylase (TH). The regeneration of L-Dopa from the spent product of the TH enzyme, dopamine, is effective only in the presence of 5HT. Our recent studies have demonstrated that Dopa decarboxylase, which regenerates L-Dopa from dopamine in the brain, is completely inhibited by the Dopa analogs, bromocriptine and lisuride. These results provide a basis for the development of a new therapeutic approach to the treatment of neurodegenerative diseases. The results also emphasize the need for a more sensitive and specific assay for TH.

900 EFFECT OF GABA-MIMETIC AGENTS AND INHIBITORS OF GABA CATABOLISM ON THE ACTIVITY OF THE VENTRAL LATERAL GEMATIC NUCLEUS (VN) IN RATS. R. L. WALTERS, S. N. LAKOSKI, M. K. HICKS, KINCS, NIH, Bethesda, MD. 20014

One model which has been used to explain effects of GABA receptors and GABA precursors on the CNS is the GABAergic hypothesis. This hypothesis states that GABAergic mechanisms are involved in the regulation of brain function. The present study investigated the effects of GABA agonists and GABA antagonists on the activity of the VN in rats. The results indicate that the activity of the VN is affected by GABAergic mechanisms. The study also provides evidence that GABAergic mechanisms play a role in the regulation of brain function.


A dense and uniform serotoninergic (5-HT) input to the amygdala (AMYG) and the ventral lateral gematic nucleus (VLG) from the midepineph, a raphe has been previously described by histofluorescence and horseradish peroxidase methods; physiological and pharmacological studies have supported these findings. The aim of this study was to determine if the serotoninergic pathway to the AMYG and the VLG has been affected by chronic serotoninergic exposure. The data indicate that the serotoninergic pathway to the AMYG and the VLG has been affected by chronic serotoninergic exposure. The data also suggest that the serotoninergic pathway to the AMYG and the VLG has been affected by chronic serotoninergic exposure.

902 MONOAMINERGIC SYSTEMS

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 our previous local and global increases in the numbers of dopamine neurons in this region (Bunney et al. J. Pharmacol. Exp. Ther. 110:569, 1975; Groves et al. Science 200:52, 1978). We have noted, however, other effects which cannot be attributed to presynaptic dopaminergic mechanisms.

Extracellular dopamine and/or related activity was recorded from chloral hydrate-anesthetized Wistar rats through single barrel microelectrodes. Cell bodies in the substantia nigra zona compacta, substantia nigra, and substantia nigra zona reticulata were identified by standard criteria and subsequent histological verification. Following recording of baseline firing rate, successive intravenous drug administrations were administered at 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 35% over baseline, an effect which was reversed by APO (see fig.).
ROLE OF THE TECTOSPINAL TRACT IN APOMORPHINE-INDUCED ROTATION

It is well known that unilateral 6-hydroxydopamine (6-OHDA) induced destruction of dopamine-containing neurons in the substantia nigra (SN) results in loss of contralateral circular rotation in response to apomorphine (Apo). The recent work of Marshall and Ungerstedt (Sci., 198, 1077) suggests that the striato-nigral projection may be established in utero. Our recent study is shown to be partly CABAergic, and its importance in rotation is further substantiated by our current finding that Apo-induced rotation can be antagonized by microcystin.

If the projection from the caudate to the SN is importantly involved in Apo-induced rotation, the question arises as to which differences 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 motoneurons 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 descends in the midbrain (the dorso lateral decussation (DDD)) interruption of this system is possible by means of a surgical knife cut made with a retractor which severs only the appropriate side.
MOTOR SYSTEMS
60 Torques were applied to dorsiflex or plantarflex the ankles of seated human subjects. The subjects were instructed to resist the torque, assist it or not to react at all.

Two phasic responses are evoked in the triceps surae and anterior tibialis muscles by a 0.05 Hz 30° rotation of the body. These responses are 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 with a latency of between 100 and 200 msec following torque onset.

The programmed response may be characterized as follows:
1) Its latency is somewhat shorter than that of a visual reaction time but comparable to a kinesthetic reaction time.
2) The latency in a single muscle is slightly but statistically different with the instructions to "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 enhance in latency. This is in marked contrast to visual reaction time 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 sole flexion and plantarflexion are a function of voluntary reaction triggered by a mechanical stimulus which is independent 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 opposite directions of the disturbing torque.

The relationship between the programmed responses seen in the leg and the apparently "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.)

912 THE CERVICO-OCULAR REFLEX IN NORMAL HUMAN ADULTS.
D.J. BARLOW* and WILLIAM FREEMAN

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 earth 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 subjects were subjected to passive head oscillations 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 average 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 peaks in the 2-D physiology 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 183 at 0.025 Hz to 43 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., reported visual fixation of a target, or attention 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 by subject. Further, three methods of analysis gain differences of up to a factor of four and phase differences ranging from 60° to 120° were observed.

The responses produced during both passive torsion 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.
B.K. Bowker* and J.D. Coulter, Marine Biomedical Institute and Dept. 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 spinal column (Burton et al., 1975a, 1975b) and the nucleus reticularis parvocellularis (Kuppers and Malicky, 1975). In this report we describe the topographical anatomy and neurophysiology of additional descending cell groups in the caudal medulla of the cat in the cervical and thoracic regions of the spinal cord and adjacent reticular formation.

Large, multiple injections of horseradish peroxidase (HRP) were made into the upper cervical spinal cord, C1-C2, cervical enlargement, C3-C4, mid-thoracic cord, T3, and lumbar enlargement. After a 3 day survival period the animals were perfused and the tissues were routinely processed and reacted with tetramethyl benzidine (Hardy and Heimer, 1977). In physiological experiments with chloralose anesthetized cats, glass microelectrodes were used to record from identified units in the caudal medulla antidromically activated from different spinal levels. Nerve fibers were characterized for these neurons.

Following injections of HRP into the upper cervical cord, medium (20-40um) and small (<20um) cells were labeled in the nucleus supraspinals and the medial nucleus reticularis ventralis (NRV) best described at the mid lumbar level and the nucleus reticularis parvocellularis (Kuppers and Malicky, 1975). In this report we describe the topographical anatomy and neurophysiology of additional descending cell groups in the caudal medulla of the cat in the cervical and thoracic regions of the spinal cord and adjacent reticular formation.

Large, multiple injections of horseradish peroxidase (HRP) were made into the upper cervical spinal cord, C1-C2, cervical enlargement, C3-C4, mid-thoracic cord, T3, and lumbar enlargement. After a 3 day survival period the animals were perfused and the tissues were routinely processed and reacted with tetramethyl benzidine (Hardy and Heimer, 1977). In physiological experiments with chloralose anesthetized cats, glass microelectrodes were used to record from identified units in the caudal medulla antidromically activated from different spinal levels. Nerve fibers were characterized for these neurons.

Following injections of HRP into the upper cervical cord, medium (20-40um) and small (<20um) cells were labeled in the nucleus supraspinals and the medial nucleus reticularis ventralis (NRV) best described at the mid lumbar level and the nucleus reticularis parvocellularis (Kuppers and Malicky, 1975). In this report we describe the topographical anatomy and neurophysiology of additional descending cell groups in the caudal medulla of the cat in the cervical and thoracic regions of the spinal cord and adjacent reticular formation.

Large, multiple injections of horseradish peroxidase (HRP) were made into the upper cervical spinal cord, C1-C2, cervical enlargement, C3-C4, mid-thoracic cord, T3, and lumbar enlargement. After a 3 day survival period the animals were perfused and the tissues were routinely processed and reacted with tetramethyl benzidine (Hardy and Heimer, 1977). In physiological experiments with chloralose anesthetized cats, glass microelectrodes were used to record from identified units in the caudal medulla antidromically activated from different spinal levels. Nerve fibers were characterized for these neurons.

Following injections of HRP into the upper cervical cord, medium (20-40um) and small (<20um) cells were labeled in the nucleus supraspinals and the medial nucleus reticularis ventralis (NRV) best described at the mid lumbar level and the nucleus reticularis parvocellularis (Kuppers and Malicky, 1975). In this report we describe the topographical anatomy and neurophysiology of additional descending cell groups in the caudal medulla of the cat in the cervical and thoracic regions of the spinal cord and adjacent reticular formation.

Large, multiple injections of horseradish peroxidase (HRP) were made into the upper cervical spinal cord, C1-C2, cervical enlargement, C3-C4, mid-thoracic cord, T3, and lumbar enlargement. After a 3 day survival period the animals were perfused and the tissues were routinely processed and reacted with tetramethyl benzidine (Hardy and Heimer, 1977). In physiological experiments with chloralose anesthetized cats, glass microelectrodes were used to record from identified units in the caudal medulla antidromically activated from different spinal levels. Nerve fibers were characterized for these neurons.

Following injections of HRP into the upper cervical cord, medium (20-40um) and small (<20um) cells were labeled in the nucleus supraspinals and the medial nucleus reticularis ventralis (NRV) best described at the mid lumbar level and the nucleus reticularis parvocellularis (Kuppers and Malicky, 1975). In this report we describe the topographical anatomy and neurophysiology of additional descending cell groups in the caudal medulla of the cat in the cervical and thoracic regions of the spinal cord and adjacent reticular formation.

Large, multiple injections of horseradish peroxidase (HRP) were made into the upper cervical spinal cord, C1-C2, cervical enlargement, C3-C4, mid-thoracic cord, T3, and lumbar enlargement. After a 3 day survival period the animals were perfused and the tissues were routinely processed and reacted with tetramethyl benzidine (Hardy and Heimer, 1977). In physiological experiments with chloralose anesthetized cats, glass microelectrodes were used to record from identified units in the caudal medulla antidromically activated from different spinal levels. Nerve fibers were characterized for these neurons.
OPERANT REINFORCEMENT OF FTG UNITS. S. M. Breedlove*, D. J. McGlynn, 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 reticular formation discharge during both waking movements and REM sleep. It was hypothesized that FTG discharge related to the motor activation component to those ataxic (Siegel and Breedlove, Science 156: 1048-1049, 1968). In this study FTG single unit discharge in cats was increased by operant conditioning using lateral hypothalamic (LH) stimulation as a reinforcer. 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 PTG 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 PTG cells, increased discharge was accompanied by an increase in motor behavior. For most of the cells, specific, stereotyped movements accompanied the increased unit discharge during the later portion of the reinforcement session. Typical movements included rotation of the head to the right or to the left, lifting the head. These stereotyped movements corresponded to the behavioral correlates of discharge determined prior to reinforcement.

In summary, unit firing in most PTG cells can be readily increased by operant conditioning techniques. These operantly-conditioned increases in PTG discharge were accompanied by increased movement, often of a specific nature unique to the cell. The increased PTG firing was not accompanied by sudden REM onset in waking or by cataplexy.


Simple step-tracking movements made by patients with unilateral cerebellar dysfunction are studied. One patient was followed for one year after the development of a unilateral left hemispheric cerebellar lesion. The second patient, with a focal left hemispheric lesion, 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 placed 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 curve 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)

SOCIETY FOR NEUROSCIENCE

916 ELECTROMYOGRAPHIC RESPONSES TO Sudden ANkle DISPLACEMENT IN NORMAL AND PARKINSONIAN SUBJECTS. D. F. Everingham, J. F. Coles, R. E. P. Wood, and 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 participants 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 Parkinsonian patients argued against its being an "a-reflex". Furthermore, the findings that the FSR latency remained unchanged in akinesic patients and that its output is proportional to input characteristics support the view that the FSR is reflexive in nature. Parkinsonian patients subjects appear 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.


Supported by the Canadian Medical Research Council.


The mammalian masseteric monosynaptic reflex is depressed during active sleep as compared to quiet sleep (Chase et al., Exp. Brain Res. 47, 1980). 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 microelectrodes (tip resistance of 1-3 MΩ) were used. During quiet sleep, the intensity of the stimulus delivered to masseter motoneuron fibers was set at a level to produce constant antidromic spike activity in 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 associated 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 which is characteristic of active sleep for approximately 10 seconds.

The reduction in amplitude of the antidromic field potential, the blockade of the antidromic spike, and the tonic membrane hyperpolarization provide indirect corollary evidence that the masseter motoneuron spike is generated at the final common pathway for the neurons of active sleep. Supported by USPHS NS 09999.

In monkeys making controlled wrist movements, corticomotorneuronal (CM) cells were identified by characteristic post-spik e facilitation (PSF) of flexion-muscle activity detected by subthreshold stimulation. Activity of these cells, which has a directly proportional effect on motoneuronal excitability, was quantified during graded voluntary movement responses. These cells were trained to alternately flex and extend the wrist between electronically detected hold zones against spring-like loads; thus, the wrist displacement was from a center position representing proportional active torques. The same monkeys also performed iso-metric ramp-and-hold torque responses, alternating between flexion and extension torques at different maxima. For each cell, response averages of cell and muscle activity, wrist position and torque were compiled for different load levels. On the basis of the 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 spikes/sec/dynes, respectively). Wrist movement waves were not found to seem equally these differences, they may reflect basic differences in the degree of cortical control of flexor or extensor muscles.

To test the participation of CM cells in postulated transcortical reflexes subserving load compensation, activity of 12 CM cells were 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 preceded the discharge of the cell but inhibition by the first post-spike (mean latency: 24 ms). The second ENG response (H2) had a mean onset latency of 30.8 ms; the difference of 6.8 ms agrees well with the measured PSP latency to cell-muscle coupling. However, half of the CM cells also showed additional responses to load perturbations inappropriate for load compensation.

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 tonic units of the medial 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 low minimum recruitment (mean: 0.00 mscs) and a high maximum recruitment (mean: 35.4 mscs) for 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 neuromuscular responses. There was a significant difference 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 histochimical studies, but rather provide an effective means of studying low threshold units.

RECRUITMENT ORDER IN THREE DIFFERENT REFLEX RESPONSES ELICITED IN A HINDLimb FLEXOR MUSCLE IN THE CAT. H.P. Clamann and A.C. Haig,* 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 Simple Principle. These properties of the reflex responses of cat tibialis anterior (TA) were studied in cats anesthetized with α-chloralose and paralyzed with Flaxedil (B). TA muscle was first passively stretched to its resting length, held in this position, 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 (3.6 mscs) latency first response, presumably monosynaptic, a short latency (-4.6 mscs) second response mediated by at least one interneuron, and a longer latency (-18.5 mscs) third response which could be abolished by spinalization, dorsal root section, or decorrelation. 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 intraxon 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 for individual motoneurons was tested in the second and third reflex 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 order of individual motor units. These results confirm previous reports from this laboratory that changes in spinal afferent input can alter recruitment order of individual motor unit in a manner similar to their size. However, when 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 NIMH.

A COMPARISON OF VOLITIONALLY CONTROLLED MOTOR UNITS IN THE GASTROCNEMIUS AND SOLEUS MUSCLES OF HUMAN SUBJECTS. M.A. Clendinend 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 superficial portion of the 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 histochimical 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 tonic units of the medial 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 low minimum recruitment (mean: 0.00 mscs) and a high maximum recruitment (mean: 35.4 mscs) for 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 neuromuscular responses. There was a significant difference 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 histochimical studies, but rather provide an effective means of studying low threshold units.

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. The 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 to the model yielded phase planes which were again qualitatively similar to those seen in the human data. The model predicted that the interaction between the model and the human could be accounted for by addition, in the model, of a force which mimicked a stretch reflex in response to the perturbation. This finding is significant for the human investigator as indicating that a major part of the limb trajectory during performance of simple movements may be determined by the limb's mechanical properties.

When the activated, afferent muscle gastrocnemius (m.g.) and soleus muscles that are stretched by physiological control appropriate velocities, their force responses are grossly non-linear. This non-linearity, termed muscle 'yield' (Nicholls and Nour, 1976, J. Physiol. 266: 449-455), of an abrupt decline in muscle stiffness that intervenes after the muscle has been stretched more than a threshold. When the muscles are subjected to similar stretches in decerebrate cats with intact stretch reflexes, the yield is no longer evident. These neuronal mechanisms of the CNS can be used to achieve this "linearization" of muscle force trajectory were investigated in this study.

A modulation of the motor output in the stretch reflex of the n.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 gms) although the number of new units recruited rapidly at high forces; the distribution of motor unit force thresholds was approximately by a decreasing 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). Neuronal recruited motor units were then observed to increase their firing rates in an exponential of 2.0 imp/sec/100 gms muscle force increase. Finally, recruitment during stretch was often accompanied by an uncharacteristically short stretch interval within the same group of motor units in soleus single and type-identified motor units in the n.g. Preliminary observations suggest that motor units recruited during stretch appear to be recruited for 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 stretch, the trajectory of the force response is strikingly similar to that of the stretch reflex in the decerebrate preparation. The force trajectroy of groups of motor units during stretch is noticeably steeper when a doublet is introduced at the beginning of the spike train. The mechanism 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.

REFLEX MODULATION DURING ONGOING MOTOR TASKS. J. R. Hirsonge and A.J. Fishburne, Jr. 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 employment and central function is unclear. This question was addressed by experiments on human subjects in which brief torque-pulse disturbances were introduced at several phases of ongoing motor tasks. "Responses" of biceps/triceps EMG activity were obtained by subtraction of perturbed and unperturbed task records. Integration of these responses revealed that the response was only slightly delayed with a high frequency component at an intermediate point. Simple proportional controllers with low gains of about 10 cause noise, unstable operation when modulating recruitment; this is not the case for a non-linear controller. Proportional controllers with low proportional gain (about 1), but with the addition of an integrator, have perfect steady state performance for this problem. The human cat can show the noise with high gain proportional controllers. Ratios of integral to proportional gain of about 10 yield the least response with no controller. This type of controller also works well with modulation of temporal summation and at the transition between modulation of recruitment and temporal summation. (Supported by NIH, NINCDS Contract Number ROI NS-2-2314).

ANALYSIS OF THE MOTOR UNIT POPULATION IN CAT FLEXOR DIGITORUM LONGUS (FDL) MUSCLE. R. P. Dunham, K. E. Burke, and J. S. Hodgson*. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

As a part of a larger study, we have examined the motor unit population in the FDL muscle of normal cats. The number and location of units examination were done using retrograde transport of horseradish peroxidase. Labeled cells were found in a narrow column about 10 mm in length dorsolateral margin of the ventral horn in the lumbar segments. A total of about 165 cells were labeled, of which 38 fell into the gamma size range (see Burke et al, J. Neurophysiology, 40:567, 1977). We examined intracellularly the size of these units and at any given time, they were in neurons and fascicles in the cat. This approach to data collection is separated into the cat's movement and the time required by the cat to move. The population 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 its lateral gastrocnemius (MG) and the number of fibers in it, is somewhat smaller 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 FDL type FR units that are classified as type S units (as compared to 23% in MG), while only 30% of FDL muscle fibers have the histochemical profile characteristic of FR units. Considering that the two tetrode units are classified as type FR units, it is not surprising that 

closed loop control of electrically stimulated muscle for orthotic purposes. Patrick F. Crago. Applied Neural Control Laboratory, Cleveland, Ohio 4411P.

Intramuscular electrical stimulation of paralyzed forearm flexors and extensors is successfully used to provide powered grasp in the hands of CS patients. A model system of human forearm muscle control would be improved if the non-linear and time-varying properties of the response to electrical excitation could be regulated. This response presents some unique problems of control and has led to the development of closed loop force feedback techniques that have been found to compensate for both non-linearities and time-dependence under isometric controller settings. The experiments were carried out in soleus muscles of cats.

A single command signal controls the stimulus parameters. In movement, the force output is increased directly from the 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 stimulating muscle fibers at a fixed amplitude and at the maximal inter-pulse interval (IPI) that allows an adequately fused contraction. However, recruitment (FR versus PFL) increases from high to low IPI's. 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 was found.

Closed loop force feedback systems have been studied with modulation of recruitment (FR) or temporal summation (IPI), and with modulation that simultaneously controlled both. Finally, recruitment at the onset of movement was observed using a non-linear controller. Control systems with low proportional gain (about 1), but with the addition of an integrator, have perfect steady state performance for this problem. The human cat can show the noise with high gain proportional controllers. Ratios of integral to proportional gain of about 10 yield the least response with no controller. This type of controller also works well with modulation of temporal summation and at the transition between modulation of recruitment and temporal summation. (Supported by NIH, NINCDS Contract Number ROI NS-2-2314).

Dopamine (DA) is localized in the atomatogastic nervous system by an indirect method along the input nerve to the atomatogastic ganglion (SGG) from the commissural ganglia (Kushner & Maynard, Brain Res. 129, 127; Barker, Kushner, & Hooper, Brain Res.in press). Electrical activation of this pathway increases the frequency of the pyloric rhythm generated in the SGG. Bath-applied DA mimic this effect with a slower time course (Anderson & Barker, Neur.102, 1982) 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 (pH). Increases in f are due to increases in the rate of depolarization since the duration of the pH is relatively constant. Simultaneous exposure to inhibitors of cyclic nucleotide phosphodiesterase would be expected to increase a DA-activated adenyl cyclase. Exposure to 40 μM DA plus 1 μM theophylline (cols. 3 & 4) enhances the magnitude of the DA response in both A and f. This enhanced DA-induced increase in the rate of depolarization is mediated by an increase in the intracellular concentration of cyclic AMP. 

**UAEEL RUNNING ACTIVITY OF RATS AFTER "PAR-LATERAL" HYPOTHALAMIC LESIONS.** W. E. Gladfelter and M. A.Mahaffy. Dallas, Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

In addition to producing a temporary aphasias and adipsia, destruction of the tuberal portion of the hypothalamus lateral and ventral to the fornix ("mid-lateral hypothalamus") produces a permanent decrease in the wheel running activity of rats killed 1 to 10 days after the lesion. Destruction of a region lying more laterally that includes the extreme lateral portion of the hypothalamus and the ventral portion of the "mid-lateral hypothalamus" also produces aphasia 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 "par-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 Vender counter. These cages were kept in a room in which the temperature was maintained at 21°C ± 1°C and the room lights were on a 12 hour on-off cycle. The rats were fed a high fat ad libitum, but the male rats were kept on a restricted feeding regimen to maintain body weight constant at 280 ± 5 g. All rats were given ad libitum. After an acclimation period of 2 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 hypothermic anesthesia (100 mg/kg) of sodium thiopental. Lesions were returned to their activity cages immediately after recovery from anesthesia and were force-fed until they began to eat and drink spontaneously. These rats 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.

The "par-lateral" hypothalamic 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 more of the motor tracts and fiber tracts within the subthalamus. This data is consistent with the hypothesis that the change in wheel running activity observed after the placement of "par-lateral" hypothalamic lesions was due to the destruction of neurons located in this area rather than to the destruction of nerve fibers coursing through this region.

**CART DESIGN PROGRAMS CONTROLLING RAPID LINK MOVEMENT IN THE CAT. G. Ochoa and D. Vitergi. Rockefeller Univ., New York, NY 10016.**

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 negative command signal, while the magnitude of the adjustment 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 characteristic of a linear function of the displacement over a large range of perturbation sizes and similar forces. The magnitudes of 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 μN. An increase in friction resulted in slowing of the response but a correct final position. Displacement of the animal's center of gravity and acceleration returned to control values in both conditions. These compensatory adjustments were accomplished by increases in the forces by the animal. The effects of the spring load both initial and terminal forces were increased. The 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. It is interesting that the magnitudes of the configuration of anticipated loads. The different adjustments in motor output in the presence of spring and frictional loads indicate that these are based on feedback control.

Slow voluntary adjustments of the ankle act in an analogous manner but fast plantar rotations involving strong phasic extensor contraction is controlled. These relationships are also characteristic of flexor responses to stretch with one important difference. The latency of the atomatic reflex in the relaxed tibial muscle is about 5 μsec and less synchronized than in the extensors. Tonic voluntary isometric contraction of the extensors increases the extensor loop gain while flexion of the flexors decreases the extensor loop gain. Both effects are proportional to the degree of contraction.

Slow voluntary rotations of the ankle in an analogous manner but fast plantar rotations involving strong phasic extensor contraction is controlled. These relationships are also characteristic of flexor responses to stretch with one important difference. The latency of the atomatic reflex in the relaxed tibial muscle is about 5 μsec and less synchronized than in the extensors. Tonic voluntary isometric contraction of the extensors increases the extensor loop gain while flexion of the flexors decreases the extensor loop gain. Both effects are proportional to the degree of contraction.
RESPONSES OF MONKEY PRECENTRAL CORTICAL CELLS DURING A CONTROLLED JAW BITE TASK. Donna S. Hoffman* and Erich S. Luschei* [SPONSOR: J.A. Ruda). Dept. Physiology and Biophysics and Regional Primate Ctr., Univ. of Washington, Seattle, Washington 98195.]


Previous work has demonstrated that phenytoin (DPH) directly suppresses discrete electromyographic (EMG) activity of muscle spindles in the decerebrate cat (Raines, JPET 191: 290, 1974). Because increased muscle tone in the decerebrate animal results from enhanced gamma motoneuron activity (Eldredge and J. Physiol., 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 decreases 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 S1-S5 were bilaterally divided. Spontaneous activity of single gamma motoneurons isolated from ventral roots L4 or L5 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 (S1-L5) was performed on 4 untreated decerebrate cats. As expected, muscle tone was eliminated in the hindlimb of the operated side.

The capacity of DPH to suppress muscle tone in the decerebrate cat appears to be related to 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 DHs' 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.

OCCISINS grantees 10667 and 12566.


The role of the stretch reflex during movement has been the subject of several interpretations. Recently Nichols & Houk (J. Neurophysiol., 49:119, 1973) have suggested that the stretch reflex regulates muscle stiffness rather than length. We applied incremental length changes to soleus, recorded the resulting changes in force, and observed a stiffness gain. Our results suggest that 1) the variability in the gain of the stretch reflex in the high decerebrate, which shows a wider behavioral repertoire than decerebrate, is due to the greater flexibility of the preparation; 2) the opportunity to stimulate 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 on soleus nerve was stimulated at 10-50Hz to generate operating forces). Reflex responses were similar in the range of 1mm-16mm to 1mm-1mm. In response to isometric 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 (mean range: 3-4). We concluded that the tendon organ feedback gain was low in this preparation.

Our results indicate that under these conditions the stretch reflex is capable of modulating stiffness in a fashion which is dependent on the point, independently of type of perturbation or changes in muscle stiffness. However, it is likely that in intact systems the stretch reflex can be modulated more adequately for increased muscle stiffness during sustained high levels of contraction. (Supported by NDA of Canada and Tech. Research Council of Denmark)


As a prelude to a study of their movement-related behavior in the conscious animal, retrograde cell labeling techniques have been used to define the distribution of cortico- and cerebellorubral cells in rhesus and cynomolgous monkeys. Horse-radish peroxidase (SIGMA VI, 30-50% solution) was injected (0.05-0.2 µL) into the red nucleus (RN) binocularly. Survival lasted from 4 days to as long as 6 weeks and the RN was sectioned at 50 µm and reacted with diaminobenzidine. A cobalt chloride modification was also used to enhance visualization of the reaction product (Adams, J.D., Neuromusc, 2: 141, 1977). All sections were examined under both bright and darkfield illumination.

Our major results may be summarized as follows. (1) Labelled cortical (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 paravocellular 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. Labelled cells were less numerous in areas 5 and 7, and very sparse in areas 3, 1 and 2. Allocortex cells were also found in the transverse dia. = 13 µ, pyramidal shaped, and situated within layer V just dorsal to the row of large pyramidal cells. Maxinum observed penetration density for these cells was only about 0.1 cell/mm3 average in layer V. (2) Only a few labelled 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 subsprinal effector afferents. (3) Cerebellar projections to the paravocellular division of the RN were derived primarily from the cortex and the inferior olivary complex (interpositus and interpolyrsumeral efferent), whereas those to the magnocellular division came principally from nuc. interpositus (ratio = 2:1). (4) Cortico-cerebellar projections to the paravocellular division of the RN were derived primarily from the cortex and the inferior olivary complex (interpositus and interpolyrsumeral efferent), whereas those to the magnocellular division came principally from nuc. interpositus (ratio = 2:1).

This work was supported by grants from the National Institutes of Health, National Science Foundation (NS 10183).
were somewhat more complex but were in general reciprocal to this suggests a contribution from a long loop reflex such as the extensors.

limb flexor responses consisted predominantly of an early excitation followed by inhibition. Responses in limb extensor 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. A later wave could be identified whose latency was shorter at higher than lower levels. This suggests that the contribution from a flexor loop reflex such as the biceps-brachii-reflex is a complex, widespread reflex response which is elicited at low forces. Therefore, although fewer units as high as 30 impulses sec.⁻¹, even at forces of units already active necessitates the recruitment of units recruited at lower forces. Mean firing rates for grouped data, where the same bouton contacted both types of dendrites.

The activities of single motor units (n=46) from human biceps brachii muscle were monitored at various forces of isometric contractions (0-888 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 888 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).

RECRUITMENT AND DISCHARGE PROPERTIES OF HUMAN MOTOR UNITS IN LOW TO HIGH FORCE ISOMETRIC CONTRACTIONS. C.C. Kukulka* and H.P. Clamann (SPON: A.J. Szumski). Dept. of Physiol., Med. Coll. of Va., Richmond, Va. 23298


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 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 decrease in the number of cristae. Eventually the electron density of the boutons increased until no fine structural details could be recognized, at which time the synapses were classified as an astrocyte and a dark process containing a high density of glia [an astrocyte and a dark process containing a high density of glia]. Very small, presumably microglia, participated in the phagocytosis of the degenerating boutons. In general the pattern of degeneration may be classified as one with filamentous type or a vesicle-containing type. The majority of degenerating boutons were located in the 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 while the nigral is 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.)

Neurons in the motor cortex have somatotopically organized, somatosensory receptive fields. However, some studies suggest that not all of the somatosensory inputs in the motor cortex arrive by way of the sensory cortex. Horseradish peroxidase (HRP) and electrophysiological techniques were used to determine if some of the somatosensory inputs 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 not in the ventral posterior lateral nucleus (VPL) also had labeled cells. Cells in the rostral VPL were 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 (μ = 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 some 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 thalamic and cortical cells. These results suggest that somatosensory inputs traveling in a thalamo-motor cortical path contribute to the formulation of receptive fields in the motor cortex.
THE UNIT ACTIVITY OF PRIMARY AND SECONDARY AFFERENTS FROM CAT HINDLIMB MUSCLE SPINDLES DURING NORMAL WALKING. Gerald E. Loeb and Jacques Davies. Laboratory of Neural Control, NIMHS, 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 conductance velocimetry criteria, we identified 21 primary afferent 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 considerably altered by a step-like change usually 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. Secondary endings 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 shakings.

Spindle activity modulation not relatable to muscle length changes was assumed to be influenced by fusimotor activity. In certain muscles (particularly those not used for an ongoing movement), this presumption leads to the conclusion that gamma motoneurons may be activated out of phase with homonymous alpha motoneurons. In other muscles, 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, alpha and gamma motoneurons may require considerable qualification. The interaction of the proprioceptive inputs from the claw with the centrally-initiated motor activity that produces movements of the crayfish claw is studied. The resistance reflex is generally thought to be inoperative during voluntary movements of the crayfish walking legs. However, there is evidence that alpha motoneurons are activated in the crayfish during voluntary or reflex activity which may be independently and independently alter the alpha-gamma relationships for a given movement. Simultaneous activation of alpha and gamma motoneurons could not be inferred under particular circumstances, but the data as a whole indicate the ability to independently control the spindles as sensory organs within their parent muscle. The 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.


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 stimulus and a target. By extending or extending his hand, the monkey maintained the cursor on the target line as the latter was step displaced or as step targets were applied to the hand. The most common type of neuron found (N=35) 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 selectively 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 NRC of Canada.


By considering movement displacement and its associated integrated ENG (IBD) as processes which go to 100%, we have derived the delta parameter for anterior deltoid increases linearly with velocity at each 10% amplitude bin and the corresponding percentage accumulation of IBD changed significantly. Surface ENG from the agonist anterior deltoid was integrated over the course of the movement and the displacement was measured by a potentiometer. Each movement was divided into a series of ten percent amplitude bins and the corresponding percentage accumulation of IBD was identified by computer. The value of the delta parameter was obtained by adding up the differences between percent IBD and percent displacement occurring at each 10% amplitude bin of the movement. Accordingly, if the IBD process goes to 100% more quickly than the displacement process, the delta parameter will be positive. Conversely, the delta parameter is negative when IBD rises more slowly than the displacement process. The delta parameter changed sign from negative to positive at different displacement velocities. However, when average accelerations were calculated for each 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 IBD with respect to movement displacement is quantitatively measurable and varies with movement speed and acceleration. The magnitude of central motorneuron control 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 prelude to a "slow" movement. The presence of positive values would be indicative of "fast" movements.
952 EVALUATION OF SPONTANEOUS FUSIMOTOR EFFECTS FROM AVERAGED DORSAL EFFECTS OF LESIONS IN DIFFERENT AREAS OF SENSORIMOTOR CORTEX ON gamma efferents, preserving the integrity of their peripheral subcortical structures which in turn mediate M2 and M3.

were sectioned. No roots were severed. While recording from the dorsal root (L 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 3b contain the essential core for 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 like M1.

(1) Tatton et al. Brain Res. 1975, 96: 108-113,

(2) Conrad et al. Brain Res. 1975, 94: 219-236,


Supported by MRC of Canada (RQ-1). A.D.M. was the recipient of an Ontario Graduate Scholarship Program predoctoral fellowship.

953 ORGANIZATION OF POSTURAL ADJUSTMENT CONTROL SYSTEMS IN HUMANS.

Lewis H. Hashman and Marjorie H. Hoollacott. Neurological Sciences Institute, Portland, Oregon 97209.

The EOG 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 the EOG and gamma antagonist activity in dorsal roots has been under­

study in this 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 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), 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) filaments were then searched for spinal afferents from the same muscle innervated by the efferent under study. An envelope of the multunit afferent discharge was obtained with a constant latency (40 msec) and averaged, with the sweep being triggered by the spontaneously occurring action potentials in the y efferent. Two types of responses correlating with fast or slow intrafusal contraction were obtained. Fast peaks in afferent response are observed at low rates of sponta­

neous activity in the y efferent, probably due to oscillations in the nuclear chain. The slower response shows maximum activity about 50 msec after the occurrence of the y spike and may correlate with contractions in nu­

clear chain intrafusal fibres. It identifies the types of y efferents contributing to the fast and slow responses ob­

served here.

REFERENCES


Single units were recorded from the left region of one monkey's precentral motor cortex during performance of a pedaling movement. The pedaling involved a reciprocal movement 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 +E 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-64053 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.

CONTROLLED LATERAL PINCH AND RELEASE IN THE C E L L S OF THE MONKEY MOTOR CORTEX. J. E. 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 tract 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 rhesus monkeys. Neafsey et al (Brain Res. 138:393, 1977) used electrophysiological techniques and found the SMA projection to the pyramidal tract and red nucleus (RN) in four rhesus monkeys using electrolytic lesions which destroyed SMA. However, they found no projection to the RN from SMA. In this study we sought neurons in the SMA projecting to the pyramidal tract (PT) and red nucleus (RN) in four rhesus monkeys using electrolytic lesions which destroyed SMA. Neurons were labeled by the antidromic invasion from stimulation of the pyramidal or red nucleus and recorded with glass coated PT/IR microelectrodes, their impulses being monitored by bridge methods. In one monkey, PT and RN were recorded with two electrodes from 0.5 to 1.5 mm. The criterion for antidromic invasion was a constant threshold and latency. The latency also being constant with high frequency stimulation; stimuli ranging from 100 to 500 Hz. The criteria for 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 A for RN neurons 401 A (the threshold ranged from 50 A to 650 A for PT neurons and 80 A to 800 A for RN neurons, with pulse duration of 0.1 msec). PT neurones with antidromic invasion from the pyramidal tract were recorded in their laminae III and the superficial parts of laminae V and VI. The PT neurons were generally found in the deeper laminae V and VI and neurons 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 or via an array of two monopolar stainless steel electrodes, 0.2 mm in diameter separated by 1 mm, with current pulses ranging from 0.5 to 13 msec. 53.6% having latencies between 0.5 and 3.5 msec. Twenty-nine projection neurons to the red nucleus were recorded and their latencies varied from 1.2 to 6.0 msec. They were more evenly distributed in their range than PT neurones. Antidromically invaded neurons from the same region in the middle brain tended to be found in the PT and RN neurones. Antidromic antidromic latencies could be recorded at one electrode site. PT and RN neurones were rarely intermingled in the same way. More RN neurones were recorded in laminae III and the superficial parts of laminae V and 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 primary motor cortex and found an average of 7.2 PT and 2.8 RN neurones per track. We found an average of 3.1 PT and 1.1 RN neurones per track in the SMA.

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, H.J. Hosko, Dept. of Pharmacol. and Anat., Medical College of Wisconsin, Milw. HI 53233

Among the indices of motoneuron-motor unit "type", an inverse relationship has been published between cell membrane resistance (Rm). Positive correlations have been established between membrane resistance and duration of after-hyperpolarization; membrane resistance and duration of total (after-explosive) contraction time (TTH) of the functionally isolated motor unit and between duration of AHP and THT. The amplitude of the maximal nonmonophasic EPSP (paired pulse depolarization CDP) for the membrane resistance. It has been assumed that the absolute voltage threshold (TH) and depolarization (DEP) necessary to elicit an orthodromic spike in the cell membrane resistance (Rm). Any cell whose Rm was less than the threshold for PT cells was 365 A; for RN neurons 401 A (the threshold ranged from 50 A to 650 A for PT neurons and 80 A to 800 A for RN neurons, with pulse duration of 0.1 msec). PT neurones with antidromic invasion from the pyramidal tract were recorded in their laminae III and the superficial parts of laminae V and VI. The PT neurons were generally found in the deeper laminae V and VI and neurons 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 or via an array of two monopolar stainless steel electrodes, 0.2 mm in diameter separated by 1 mm, with current pulses ranging from 0.5 to 13 msec. 53.6% having latencies between 0.5 and 3.5 msec. Twenty-nine projection neurons to the red nucleus were recorded and their latencies varied from 1.2 to 6.0 msec. They were more evenly distributed in their range than PT neurones. Antidromically invaded neurons from the same region in the middle brain tended to be found in the PT and RN neurones. Antidromic antidromic latencies could be recorded at one electrode site. PT and RN neurones were rarely intermingled in the same way. More RN neurones were recorded in laminae III and the superficial parts of laminae V and 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 primary motor cortex and found an average of 7.2 PT and 2.8 RN neurones per track. We found an average of 3.1 PT and 1.1 RN neurones per track in the SMA.

SOCIETY FOR NEUROSCIENCE
The relation between extrafusal and intrafusal activation in the decerebrate cat model is presently unclear. In some cases (such as isometric contraction of human finger flexors (1)) the activation appears to be triggered by innocuous mechanical stimuli. In contrast, in others (such as isometric contraction of human finger flexors (2)) the relation is claimed to be less rigid. Furthermore, the physiological role of beta axons, whose presence is now widely accepted, varies from one muscle to another, as indicated by the results of some studies.

In most primary endings (3/42) crossed extensor activation induced a progressive increase in receptor discharge, commencing well before the onset of extramuscular force or any activity. There was usually no further increase in discharge once extramuscular 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 extramuscular threshold.

A smaller number of primary endings, and a significant fraction of secondary endings, have been detected (4). In these cases acceleration of discharge was mediated via a different class of gamma fibers to that cited above, it is equally likely that beta motoneuronal activation 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.


The purpose of this investigation was to characterize, at the electron microscopic (EM) level, the fine grain degeneration seen in the ventrolateral-posterior (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. The state of intrafusal activity was deduced from the discharge rates of identified primary and secondary endings, isolated from small dorsal root filaments. Spindle receptor properties were examined using isometric conditions, during isometric muscle stretch and during isometric 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 extramuscular force or any activity. There was usually no further increase in discharge once extramuscular 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 extramuscular threshold.

A smaller number of primary endings, and a significant fraction of secondary endings, have been detected (4). In these cases acceleration of discharge was mediated via a different class of gamma fibers to that cited above, it is equally likely that beta motoneuronal activation 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.
962 PATTERNS OF CONTRALATERAL LIMB RESPONSES TO NOCICEPTIVE STIMULI DURING LOCOMOTION. S. Rossignol and L. Gaunttice*, Centre de recherche en sciences Neurologiques, Universite de Montréal, Québec, Canada H3C 3T8.

When a noxious skin stimulus is applied on a skin nerve of a hind limb in cats treated with phenolization, the contralateral hind limb extends where it is placed initially in a flexed position and conversely flexes when placed in extension (Grillner and Rossignol, Brain Res., 144: 411-421, 1978). The purpose of this study also reports on a reversal of contralateral effects but during locomotion in high decerebrate cats walking on a treadmill. The stimulus (100 Hz trains, 100 µA, pulse width of 300 µsec, 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 somewhat prior to the burst and moderated over 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's extensor burst amplitude increases 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 rephrase the next properly the other limb. This work emphasizes that with a nociceptive stimulus the classical reflexogenic and the second psychogenic, involved in the modification of R1 and R2. The plasticity of R1 and R2 was investigated as a function of sensory stimulation preceding reflex elicitation. We note that prior experiments which have reported facilitation of R1 and inhibited R2. Facilitation of R1 developed more rapidly than did inhibition of R2. The time course of facilitation that the dynamic force trajectory in our subjects may rely largely upon intrinsic muscle properties. The amg response may occur at a dominant frequency of about 7/sec, appear during exploratory sniffing behavior, and are of large amplitude. The alpha-tremor activity of septal lesions, which disrupt exploratory vibrissa movement and theta waves. We are currently investigating the effect on the alpha-tremor activity of septal lesions, which disrupt exploratory vibrissa movement and theta waves. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in 3 rats.
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 ±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).


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.

A single motor unit was functionally isolated from ventral rootlets at the L5-L7 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 behavior of the afferent response during the motor unit contraction changed qualitatively and quantitatively as additional motor units were activated. This relationship between the time course of the afferent PST histograms and each averaged motor unit twitch record was modified as additional independently stimulated motor units were activated.


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. 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). Stimulation threshold was set at 4x threshold for perception. Such stimulation is not painful but elicits a sensation similar to having the finger firmly pinched. With one exception, the recruitment 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 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 20 to 180 g (or approximately 6% maximum; n = 17) had their recruitment threshold raised by skin stimulation.

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 disturbances of a stable system to continuous internal disturbances. According to R.W. Jones (Principles of Biological Regulation, Academic Press, NY, 1973, pp 245-246), repeated oscillations of 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 mean frequency of 6/4 sec, root-mean-square (rms) displacement amplitudes ranging from 30 to 100 microns. Hand tremor 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 16/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 is a high-order system. High coherence values were obtained between the amplitude demodulated EMG and hand tremor for amplitudes above about 100 micro. 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 consistent with the postural hand tremor is not sufficient for the maintenance of these stable oscillations. (Supported in part by USPHS Grant NS-08692.)

NUCLEUS OF THE CRUS CEREBRI (NCC): A PREVIOUSLY UNDISCOVERED CELL GROUP FORMING A RETICULUM AROUND MOTOR SYSTEM FIBERS DERIVED FROM THE CEREBRAL CORTEX AND MIDBRAIN. Konrad Talbot (1) and Larry L. Butcher (1,2) Dep. of Psychology (1) and Neurosurgical 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 caudal mediodorsal tip of the subthalamic nucleus (SN,md) and the rostro lateral 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 distinguished from cells of the nigra. NCC neurons are seen to be distributed as single layers of intensely stained somata and processes, especially at caudal levels, to form a reticulum through which course bundles of motor system fibers. The morphology of the NCC neurons is such 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 caudal and rostro-cortico-lateral neostriatum to the SN,pc. Although gaging mechanisms come to mind, further work is clearly required to elucidate the 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.)


The monosynaptic connections of jaw elevator muscles to motor neurons 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 18 µV. 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 multisyaptic 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 1A antagonist inhibition of jaw muscles, these interneurones could be excitatory to the motoneurones. Secondly, 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 synaptic noise reflects 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 NS02957.)

Four monkeys were subjected to unilateral lesion of dorsal column nuclei (DCN) and four to dorsal rhizotomy C2-T2 (DR). Post-operative behavior was monitored up to 18 months postoperatively. Movement of forelimbs and hindlimbs compared on two tasks. One task required limb movement forward into a cylinder. Latency from presentation of the container usually required entry was about 15 sec. One to two sec from entry to grasp and withdraw with food was a measure of coarse grasp. The second task required reaching and grasping a food pellet from one of three positions along a narrower corridor. This task yielded measures of fine reach and grasp as well as allowing detailed evaluations of the movements involved.

With DCN lesion various results were observed. The main result was a transient involvement of proximal limb musculature. After 6 to 8 weeks postoperatively, only transient involvement of proximal limb musculature. After 6 to 8 weeks postoperatively, there was 'no' involvement of distal musculature. After another month, there was a chronic deficit in coordinated finger movements involving all fingers or independent scooping by thumb or index. When permitted to test, there was a significant delay in the time that 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 intact contralateral limb. The delay was greater than the lesioned 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 proximal to distal recovery seen after DR in this and previous studies. After DCN lesions there is chronic deficit in coordinated finger movements with only transient involvement of proximal limb musculature. After DR, fine finger movements involve the thumb alone. 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 byafferent input ascending via DCN. Thus a distal deficit predominates. DR interrupts gamma loops through which non-pyramidal influences operate. The pyramidal system is free to recover its control over distal musculature. Supported by NIH Grant #2 R01 NS12330 to A.J.B.

EFFECTS OF FOCAL INJURY IN AREA 4 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 if physiological changes occurring subsequent to focal injury in area 4. The fundamental question investigated in these experiments was: Do injury related changes in sensorimotor function reflect the limitations of the motor cortex, or are mechanisms intrinsic to the cortex or to subcortical systems which contribute to cortical neuron excitability? To answer these questions, two groups of cats were exposed and covered with a closed chamber. Bipolar concentric electrodes were inserted into the contralateral dentate nucleus, in the ipsilateral medullary pyramidial tract, and the ipsilateral lateral spinothalamic tract. A bipolar concentric electrode was also placed in the posterior intermediate setulus 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 of the late cerebellar nucleus elicited by dentate stimulation was reduced by 50-60% for 3 hours following the production of the lesion. However, in the same region of the cortex, 30-50% of the same evoked activity was elicitied 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 can modulate function of subcortical systems which contribute to the excitability of the motor cortex. (Supported by The American Heart Association, Suncoast Chapter, 7/77 AG 613)


We have previously shown that rapid isometric responses made by cats tracking sudden target perturbations, using a compensatory display by their force, are controlled by a pulsectile neural signal of constant duration and variable amplitude (Ochs 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. Therefore, the force can be scaled from their onset and the peak of the force derivative (dp/dt) was predictable 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 target.

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, when mechanical delays and reflex effects are minimized. Latencies in this task are very short; most daily latencies measured from the perturbation to the onset of dp/dt were 60-80 msec. The peak dp/dt is reached after another 30-55 msec.

When the second perturbation preceded the peak dp/dt (of the response), the cats could predict the changes in the neural output and required a reduction in force applied, the peak dp/dt decreased progressively with shorter ISIs. Peak force levels were proportionally reduced. The cats were sensitive to its rectification. The amplitude of the shortest ISIs (10-20 msec), though in such cases EMG activity of remote synergistic muscles was still occasionally present. Accompanying these changes in amplitude, there was a decrease in the time from onset to peak dp/dt, indicating that the output pulse had been shortened in its duration. These data, obtained under isometric conditions, suggest that the absence of reflexes is likely, that the sampling models which imply central refractoriness. The timing of the observed corrections in ongoing motor output suggests that responsiveness may be due to a segmental as well as sensory information with no detectable increase in reaction time. Supported by Grant NS 12730.
**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 held in the hand while placing the arm in a 90° flexed position against a range of steady-state torques. Stimuli given at pseudo-random intervals were of two kinds: (1) 50 ms DC torque pulse (TP), flexion or extension, 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 an in vivo force via an external electromagnetic coil snugly embedded in the distal musculotendinous junction (Wolpaw and Colburn, Br J Surg 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.

Two monkeys (Macaca mulatta) 307 units in areas 4, 3a, and 4, and 1 responded to flexor and/or extensor TP, the primary sensori-motor cortex (SMC) is a complex cortical region which may exist in the motor cortex of the rhesus monkey. There is a double representation of the hand in area 4 of the motor cortex, particularly evident for neurons in layer III, where large 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 laminar pattern. In contrast, most callosal neurons in the somatic sensory cortex are found in layer III and only occasional callosal neurons are seen in layers II and IV. Second, the mediolateral strips of labeled neurons which are prominent feeding the somatic sensory cortex (Jones, et. al. Science 190: 572, 1975) are not as distinct in motor cortex. Third, many more callosal neurons are found in the arm motor area in layer III 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 containing 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 of the somatic sensory cortex are in need of reexamination in light of these new findings. (Supported in part by funds from the Veterans Administration Medical Research Service and Department of Neurosciences, Upstate Medical Center).
NEUROCHEMISTRY
Recently a method has been developed to isolate neurons from adult rats which retain a reasonable part of their processes. (Altman, Dept. Neurobiol. and Anat., UTMS, Houston, Texas 77025). This study was supported by NIH-NINCDS Grant No. NS11066. 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 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 phosphohydrolases (EHP) 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 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).

**Effects of Chronic Antipsychotic Drug Treatment on Dopamine Metabolites in Primate Brain.** N. G. Bacopoulos*, D. E. Redmond, J. Baumb* and R. H. Rotta. 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 6-7 months to female fetal rhesus 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 animals were injected with 1.0 mg/kg haloperidol i.u. (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 gas chromatography. 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. Chronic pretreatment with haloperidol enhanced the HVA-elevating effects of the acute challenge dose in dorsal frontal, orbital and cingulate cortex. In the temporal, septal and occipital cortex, the acute challenge dose had the same effects on HVA concentration in chronically pretreated and previously untreated control animals. HVA was quantitated primarily the metabolites 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 10-100-molar haloperidol was 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.)


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% O2 and 5% CO2. 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 h 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 0.2M sucrose. Purified myelin was prepared according to Wigg et al. (Brain Res. 99, 1975). In the temporal, parietal and occipital cortex, the acute haloperidol (mg/kg haloperidol i.m. (acute challenge dose) control 1.12 ± 0.08(5))

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control</th>
<th>Degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.15 ± 0.18(9)</td>
<td>3.50 ± 0.12(8)</td>
</tr>
<tr>
<td>3</td>
<td>0.75 ± 0.10(5)</td>
<td>1.22 ± 0.16(9)</td>
</tr>
</tbody>
</table>

As can be seen from the data, no significant increase or decrease in the myelin synthesis was observed for 1 day for 3 days after nerve degeneration. By 3 days post transection, however, myelin synthesis appears to be increased by about 30% in response to injury. At 7 days after section, myelin synthesis of the degenerated nerve degeneration, and myelin synthesis has decreased by 45%. (The high value at 5 days for the contralateral control results from a single sample; by omitting this number one gets a mean of 1.12.)

**Biochemical Characterization and In Vitro Maintenance of Neurons from Adult Rat Brain Isolated by the Perfusate Method.** H.B. Althaus*, W.B. Huttner*, K.P. Gebicke*, V. Neuhoef* and J.D. Lane, Max-Planck-Institut für exp. Medizin, Forschungsstelle Neurowissenschaft, 3400 Göttingen, FRG.
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. 48:53A, 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 3H-valine from one animal were combined with 14C-labeled cytoplasmic or serum proteins from another animal. The mixture was separated electrophoretically on gels, which were then cut and counted for 3H and 14C. High 3H:14C 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 ß and γ (see Abstract: Shashoua, this volume), proteins whose metabolism is altered by training (Shashoua, Science 193:1264, 1976). The ß 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 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.)

WITHDRAWN BY AUTHOR


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 mc of 32P-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 corticyles 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 [y32P]ATP as described previously (Mullinan, Wilson & Williams, Bioch. 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 laid across 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 32p incorporation into PSD proteins was observed following the in vitro 32P-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. This also opens the possibility that the postsynaptic density-phosphorylation state of these proteins may play a role in their suggested function as modulators of synaptic transmission (Bienenstock & Siegelkutz, J. Cell Biol., 1977, 74, 204-225). (Supported by NIH Grant NS-07457.)


Silastic Pudenz catheters were chronically implanted in the fourth ventricle of monkeys and were connected to compressible polyethylene Omaya 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 hemiepithrocytic biopsies of cerebral and cerebellar cortex samples 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.
High performance liquid chromatography (HPLC): New insight into cyclic AMP metabolism. Major contributions to cyclic nucleotide phosphodiesterase (PDE) from catadine 5'-monophosphate (c5AMP). Subsequently, 5'AMP is phosphorylated to ATP by a further metabolic reaction catalyzed by the PDE. We report that an alternate pathway, 5'AMP may be deaminated to inosine monophosphate (IMP) and converted to inosine by the catalytic 5'-nucleotidase. The conversion of inosine to hypoxanthine then proceeds via the xanthine oxidase pathway. We are now reporting a new HPLC technique by which we separate, identify, and quantify cAMP and its metabolites in vitro. The reagents for these techniques are: 1. TABs, 2. TMS, 3. TFA, 4. AER.

We also report the development of new HPLC techniques for the separation of metabolites of cAMP and cGMP. These new procedures include: 1. A new method for the separation of cAMP from cGMP. 2. A new method for the separation of cGMP from IMP, XMP, and GMP. 3. A new method for the separation of IMP from XMP and GMP. 4. A new method for the separation of XMP from GMP.

Neurochemistry

Central myelination in vitro: Effect of reduced calcium and magnesium. William Crevelo, Jack Millonig, and Francis A. Thomas. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

It has been suggested that deficiencies in calcium and magnesium experience during infancy may lead to improper development of myelin (Goldberg, 1974; Intern. J. Dev. Stud., 6, 121). This hypothesis has been tested in vitro using slices of spinal cord from 10 to 12 day old chick embryos. Control media contained approximately 2.0 mM Ca and 1.0 mM Mg, and test media was identical except for a moderate reduction in calcium and magnesium produced a noticeable deficiency in myelin growth. Since these results suggest that there may be certain minimum levels of calcium and magnesium that promote adequate myelination. Since these results suggest that there may be certain minimum levels of calcium and magnesium that promote adequate myelination.

The optimal ionic requirements necessary for the proper growth of myelin have not been explored previously, and these requirements may be physiologically relevant. As suggested by Goldberg, the demyelination experienced by patients with multiple sclerosis may be related to a result of improper early development of myelin, due to deficiencies in calcium and magnesium. Published lipid analyses of myelin from multiple sclerosis victims have found developmental abnormalities, most of which is increased in the fatty acids, particularly in cerebroside and decreased fatty acid chain length (Nolke & Borri, 1973; Europ. Neurol., 10, 750; Peetwar et al., 1976; J. Neurol. Sci., 25, 129, 1976). 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 presented results suggest that deficiencies in calcium and magnesium could be responsible for the developmental errors.
We have reported an unusual case of mannosidosis (Kissler 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% was available for us to analyze. 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 precipitated with ethanol, 2-3 M. The molar ratio of mannose to glucose isolated from mannosidosis urine. Carbohydrate analysis of the crude fractions from Biogel P-2 by GC-MS revealed that fraction 1 contained a single glucose component with a molar ratio of mannose to glucose that was presumably below 1. Fractons 3-6 each contained mannose and glucosamine together with small amounts of glucose. The molar ratio of mannose to glucosamine in fraction 1 was determined to be 5, 3, and 2 respectively. Control tissue (4.5 g) obtained from hyperplasia. The gingiva was infiltrated with histiocytes containing no components corresponding to the mannose containing sugars. The sole ergone positive peak in the control co-eluted with fraction 1. The presence of rat striahtal slices; nialamide was included in the medium at all times and appeared to inhibit MAO. However, in release studies, nialamide became effective only when the MAO was low and the dominant metabolites were being washed into the bathing medium. Degeneration occurred in the decreasing order: p-TA> m-TA> DA. In order to determine whether the differential selectivity for degeneration was due to an innate substrate selectivity of MAO, a substrate selective inhibition by nialamide, or a substrate protection from MAO by intraneuronal storage; the ability of rat striahtal homogenates to deamininate p-TA, m-TA and DA were assessed with and without the addition of MAOI's. Their effect on uptakes was also assessed.

MAO activity of striatal homogenates was assayed by measuring the decline in amine substrate concentration and the production of acid-soluble metabolites. Preliminary studies showed that 20 min, 10 μM MAOI and 3 min incubation (37°C, 1 μM MOAI, 10 μM tritiated amine) were performed at 37°C. After the incubation, the amines were extracted into diethyl ether by phase separation. The phosphorus-containing amino acids were subjected to alkali hydrolysis and the amino acids were estimated 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 40% of p-TA, m-TA and DA, respectively. The efficacy of the MAOI's in inhibiting MAO with all amines, did not interfere with their uptakes and they therefore appeared to be suitable for use in transport studies. Their effect on uptakes was also assessed.

MAO activity of striatal homogenates was assayed by measuring the decline in amine substrate concentration and the production of acid-soluble metabolites. Preliminary studies showed that 20 min, 10 μM MAOI and 3 min incubation (37°C, 1 μM MOAI, 10 μM tritiated amine) were performed at 37°C. After the incubation, the amines were extracted into diethyl ether by phase separation. The phosphorus-containing amino acids were subjected to alkali hydrolysis and the amino acids were estimated 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 40% of p-TA, m-TA and DA, respectively. The efficacy of the MAOI's in inhibiting MAO with all amines, did not interfere with their uptakes and they therefore appeared to be suitable for use in transport studies. Their effect on uptakes was also assessed.

Several recent studies have indicated that the ability to measure blood levels of a putative neurotransmitter 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 the methods which were 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 radioactive 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-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 radioactive assay it was found that blood GABA content is elevated 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 pmol/ml in 8 mammalian species with human values averaging about 900 pmol/ml. In vivo administration of GABA 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 effects of GABA with the in vitro studies 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 USDA grants RDA No. NS-00335 (S. J. E.) and B-27938.)


Following the injection of tritiated amino acid a large portion of brain soluble radioactivity is in the form of tritiated water (THO), and show here that the portion increases with postnatal age. Long Evans female rats at various ages were injected intraperitoneally (IP) with L-(4,5-3H(N))-leucine (50uCi/100g body wt.). After 5 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% trichloroacetic acid (TCA). The THO 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 counting. The other aliquot was dried under nitrogen at 50°C and resublimated in the original volume of water before determining the evaporative loss of radioactivity (THO). The THO percentage of total radioactivity, that is, the percentage of radioactivity lost through evaporation of the acid soluble fraction was:

\[
\text{THO} = \text{SD}
\]

<table>
<thead>
<tr>
<th>AGE (d)</th>
<th>CEREBRAL CORTEX</th>
<th>LIVER</th>
<th>BLOOD</th>
<th>SCIATIC NERVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.0±0.49</td>
<td>7.50±0.87</td>
<td>9.47±0.75</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>13.5±1.54</td>
<td>6.0±0.48</td>
<td>9.1±0.28</td>
<td>8.0±0.82</td>
</tr>
<tr>
<td>15</td>
<td>17.0±0.82</td>
<td>7.0±0.37</td>
<td>14.1±1.5</td>
<td>6.0±0.97</td>
</tr>
<tr>
<td>20</td>
<td>22±1.8</td>
<td>9.1±0.6</td>
<td>15.1±1.0</td>
<td>11.0±1.0</td>
</tr>
<tr>
<td>25</td>
<td>30±1.4</td>
<td>12.1±1.3</td>
<td>16.3±1.3</td>
<td>17±1.0</td>
</tr>
<tr>
<td>30</td>
<td>37±2.1</td>
<td>12.1±1.7</td>
<td>19.2±2.2</td>
<td>14.3±3.2</td>
</tr>
<tr>
<td>ADULT</td>
<td>45±1.6</td>
<td>14.0±6.0</td>
<td>25.1±2.6</td>
<td>28±3.1</td>
</tr>
</tbody>
</table>

The proportion present as THO is either constant or increases slightly during the first two postnatal weeks. However, during the third and 4th postnatal weeks, THO values exchanged from 40 to 10 days are comparable to those for leucine. At least for the case with leucine, the percentage of radioactivity present as THO was the same in brain, cerebellum, cortex and medulla. This work was supported by U.S. Public Health Service Grant NS-14355.


Two tetrahydroisoquinoline derivatives, 3'-O-methylnorlaudanosolinecarboxylic acid (NLCA) and 3'-O-methyllaudanosolinecarboxylic acid (DNLCA) have been shown to be potent inhibitors of the conversion of dopamine and 3'-4'-dihydroxyphenylalanine, its methylated product, MNLCA is elevated upon administration of L-dopa (C.J. Consolati et al., Nature 259, 617, 1977). To determine the effects of MNLCA on enzymes of catecholamine metabolism in vivo, a preliminary investigation was undertaken using rat adrenal medulla explants maintained in culture. In preliminary trials it was demonstrated that tritiated NMLCA, NLCA as well as dopamine were taken up by medullae. At the end of the incubation concentrations of the NLCA's 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 NLCA, DNLCA or NLCA and [3H]-dopamine. After 12 h the medulla were removed from the media, carefully washed and homogenized. Upon addition of carrier catecholamines and their various metabolites, the mixture was subjected to paper-2D reversed-phase high pressure liquid chromatography which afforded complete resolution of the components examined (M. Mitchell and C. J. Consolati, 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 concomitantly, levels of dopamine catabolites (3', 7-O-dimethyl derivative. Thus both in vitro and in vivo evidence suggest that the effects of NLCA will depend on the extent of its methylolation. (Supported by NIH Grant NS 12342.)


Piperic acid, an iminocacid related to l-tyrosine metabolism, has recently been identified in the mouse brain in our laboratory by means of TLC and mass spectrometry. Endogenous levels of 184 nmoles/g piperic acid were found in whole brain homogenates of adult mice. Several organs of the adult mouse, including brain, showed in vitro formation of piperic 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 piperic acid from l-lysine was studied in whole embryonic brain and in vivo studies showed that the brain formation of piperic acid could be detected as early as 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 [3H]-piperic acid at a concentration of 2×10^-3 M. The uptake was Na+ dependent, ouabain sensitive and showed at Km=4.7±10^-4 M. Structural analogues of piperic acid showed a significant inhibitory effect on uptake at a concentration of 10^-5 M. Release of piperic acid could also be demonstrated in rat brain slices. The demonstration of presence, biosynthesis in adult and embryonal brain and high affinity uptake of piperic acid, suggests a physiological role of this substance in the central nervous system of the mouse.

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, and that the synthesis is also present in rat Harderian gland and retina besides other tissues. A product of the enzymatic reaction has been characterized as the 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-adenosylmethanetransferase is not elevated above the brain, and one that of the liver enzyme. Km for L-methionine and ATP has been determined as 6.2x10^{-5} and 6x10^{-4} M respectively. Magnesium at a concentration 25 µM is catalytic for S-adenosylmethionine, higher concentrations are found to be inhibitory. 50 mM potassium is also present in rat Harderian gland and retina besides other tissues. These findings suggest that choline stimulates central cholinergic function.

1003 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, 146 (1977)], and alleviates the symptoms of cardiac 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 acetylcholineesterase 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 to rats (10 mmol/kg, ped rats) enhanced the rate of depletion of striatal dopamine induced by simultaneous administration of a-methyltyrosine (a-MT, 1.2 mmol/kg, i.p.), a tyrosine hydroxylase inhibitor (a-MT alone=50% decrease; a-MT with choline=68% decrease measured 1 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 mmol/kg, 1 hr apart). Administration of physostigmine sulfate (6 mmol/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 mmol/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-napthylvinyl)pyridine (NVP), (0.7 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.
INVOLVEMENT OF LSD-INDUCED HYPERThERMIA IN THE DISAGGREGATION, OF RABBIT BRAIN POLYSOMES. John J. Heikkila and Ian R. Brown

1006 Dept. of Zoology, Scarborough College, Univ. of Toronto, West Hill, Ontario, Canada, NIC 1A6.

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 occurs within minutes after injection, whereas brain temperature within minutes after injection, whereas brain temperature increases (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 µg/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 cause brain polysome disaggregation. Rabbits given 25 µg/kg of LSD showed marked elevations in rectal temperatures measured in conjunction with the examination of brain polysome profiles 1 hr after LSD (100-1000 µg 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.

1007 REGULATION OF AERobic METABOLISM IN STIMULATED GARFISH OLFACTORY NERVE. L.A. Hersey, S.H. McDougal, R.V. Dargar* and D.B. McDougal* (SPON: Enoch Callaway)

Regulated through the ATP/ADP-Pi 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.

THE ROLE OF pH IN THE ISOLATION OF NERVE ENDING PARTICLES WHICH TRANSPORT GABA. Robert J. Hitzemann* (SPON: Enoch Callaway)

Departments of Pharmacology and Psychiatry, University of California, San Francisco, California 94143.

Nerve endings were prepared from the whole rat brain using sucrose and sucrose-Hepes buffer solutions. The grade of sucrose used to make the homogenizing solutions 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 S-H. The gradient was centrifuged for 45 min x 83,000 g. Slices (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 [3H]GABA transport appearing in the combined five gradient fractions. Even increasing the pH from 7 to 7.8 significantly increased [3H]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 [14C]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 from 7 to 7.8 did not significantly increase the content of fraction C. Finally, it was found that increasing the S-H pH increased the total number of [3H]GABA 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.

Oxidative metabolism was shown to be necessary in supporting the unreacted labeled acetyl-CoA leaving products in the supernatant. Whole blood, plasma and RBC from mammals including humans, rabbits, monkeys, rats and mice were homogenized in equal volumes of 0.5% Triton X-100 and 10% volume of 3M NaCl. Enzyme activity influences such a transition. Garfish nerve was chosen for this study because it is a myelinated nerve. Metabolism of neurotransmitters is involved in the disaggregation of rabbit brain polysomes. Increases of hyperthermia in the LSD-induced disaggregation of rabbit brain polysomes.

(Continued from the Medical Research Council of Canada)
DIRECT PHOSPHORYLATION OF TYROSINE HYDROXYLASE BY cAMP-DEPENDENT PROTEIN KINASE: A MECHANISM OF ENZYME ACTIVATION. T.H. Joh, D.R. Park, M.J. Brodsky*, and D.J. Reis, Laboratory of Neurobiology, Cornell University Medical College, New York, N.Y. 10021.

Over the past several years the demonstration that the activity of a specific enzyme can be regulated by phosphorylation 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 the answer: (a) if TH in the 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), On-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 found for rabbit liver MAO. Some differences were noted, i.e., TH in brain and adrenal medulla is not phosphorylated by PK.

We have shown that the extraction of 45Ca 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 Ca across the brain-CSF interface (Soc. Neurosci. Abstr. 3:111, 1977). To investigate the possibility that this effect might also exist at the cellular membrane level, we have studied the uptake and efflux of 45Ca in vitro in Pb poisoned 30-day-old albino rabbits. The clearance of 45Ca from CSF, measured by ventriculo-cisternal perfusion, was significantly 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 45Ca across the brain-CSF interface. In Pb poisoned animals receiving 165 mg of Pb(CO3)2 daily for 5 days, no significant difference was noted in the rate of clearance of 45Ca from the ventricle. The uptake of 45Ca 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 0.63 ± 0.08 for controls and 0.86 ± 0.12 for brain cortex exposed to lead nitrate (5x10-5M) (DES). For the study of 45Ca efflux, brain cortex, paraventricular tissue (PVT) and choroid plexus (CP) slices were rapidly removed and incubated for 30 min in a medium containing 45Ca 350,000,000 cpml/mg (sp. act. 17.9 mCi/mg). The slices were washed in cold artificial CSF, transferred to unlabeled CSF at 37°C and incubated for different periods of time (15 to 60 min). The uptake of 45Ca into CP at 5 min was increased 75% in animals treated in vivo with lead carbonate (P<0.01), 69% in tissues incubated in vitro with 2-(p-amino-ethyl)monoethylamine (P<0.01), and 483 with Sodium Azide (300) (P<0.05). However, such treatment did not increase 45Ca 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 proteins in the process of active transport. A resultant imbalance of intracellular Ca levels may influence Pb Neurotoxicity. Supported by a grant from the NIH ROI-E501151.


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. 52:282-178, 1978, Brain Res. 142:71-78) suggest that those 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 undergraded 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 Sg and 18 Sg. There was no apparent 5 Sg class and the 4 Sg class was disproportionately large compared to that of brain tissue. In addition, axonal extracts contained an apparent 15 Sg 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 Sg 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.
1016 PURIFICATION OF CHOLINE ACETYLTRANSFERASE FROM CHICKEN BRAINS.
Kelvin Ma* and S.C. Song, 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 hydroxypatite, 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 chromatography 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 MM of the major band was 63500. The lack of carntine acetyltransferase in the enzyme preparation was indicated by the low specific activity of carnitine acetyltransferase of 0.17 moles acetylcarnitine formed/min./mg. protein compared to 24 moles/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 (10% 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 MRC).

NG108-15 cells possess many neuronal properties, including the ability to form neuropeptidergic synapses in culture following prolonged exposure to dibutyryl cAMP (Bt2cAMP). Previous experiments have shown that cells grown in the absence of Bt2cAMP are unable to release acetylcholine in response to depolarization but gradually develop this capacity during Bt2cAMP treatment. Since the ability to accumulate choline is essential for the functioning of cholinergic neurons, the choline uptake system of NG108-15 cells and the effects of Bt2cAMP on these systems were studied. Cells grown in the absence of Bt2cAMP exhibit a 2 choline uptake system: a high affinity system, Km = 3.4 µM, Vmax = 160 pmol/min/mg protein; and a low 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 Mg++. A tenfold elevation of Ca++ or Mg++ 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 synaptosomes, a high concentration of hemicholinium-3 (50 µM) was required to cause 50% inhibition of the high affinity uptake system (0.5 µM choline).

Choline uptake in NG108-15 cells cultured with 1 µM Bt2cAMP 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, the 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 changes in kinetic parameters following Bt2cAMP treatment are involved in the development of depolarization-dependent release of acetylcholine by these cells remains to be determined. However, 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 neurablastoma synaptosomes, the results suggest that the Na+-dependent uptake system is not required for synaptic function. An alternative explanation that cannot be ruled out at this time is that this presynaptic uptake 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 choline uptake system. (Supported by BRS RR05656 and the PMAF.)


Injecting CH3HgCl in low doses to pregnant rats on the 4th day of gestation results in inhibition of the incorporation of label from (DL)-3-OH-[3-14C]butyrate, but not from [1-14C]glucose, into total extractable brain lipids during the period of acute 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 3.64 ± 0.5 µg/g, 5.71 ± 0.8 µg/g and 0.44 ± 0.1 µg/g, respectively, on postnatal day 1; and 12.1, 11.8 and 3.3 µg/g, respectively, on postnatal day 21, the asymptomatic dams' brains. These data are consistent with evidence of others that the fetus may be more susceptible to methylmercury intoxication than is the mother, and that 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 cholines and phosphatidyl serine than from [1-14C]glucose, into total extractable brain lipids during the period of acute myelination (day 74), 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 before butyrate uptake by brain slices and its conversion to acetyl- and malonyl-CoA to lipids and (b) inhibition of the conversion of cytidine diphosphocholine to phosphocholine by DTPA and either inhibition of the conversion of cytidine diphosphocholine to phosphocholine by DTPA. Further experiments are planned to elucidate the detailed mechanism of this inhibition. (Supported by NIMH Fellowship 1 F31 MH07533-01.)
Wave radiation (3.0 sec, 2.5 Kw) 2 min to 24 hrs after initiation of band F, rapidly labeled in vivo and affected by experience may be an orthophosphate into neostriatum were sacrificed by focused micro-

fications. An increase in band F phosphorylation was found in an 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 signifi-
cantly greater in vitro phosphorylation of band F than the un-

PHENYLACETIC ACID IN BLOOD AND CEREBROSPINAL FLUID. Aron D.

Evidently, the axosomes provide a unique model for the study of biochemical events related to electrogenesis. Some of the special features of this experimental preparation for bio-

chemists is that the sheath can easily be separated from the axon. In the present studies, we have analyzed the labeled proteins in axoplasm by the polyacrylamide gel electrophoresis and autoradiography several

synaptosomal phosphoproteins (bands D1, G (~34K), and H1,2,3 (~18-13K), using polyacrylamide gel electrophoresis and autoradiography several hours after injection of P32-P-ATP for the analytical difficulties found in measuring these substances in bio-

logical fluids. We now report the presence of phenylacetic acid (PAAC) in rabbit and human cerebrospinal fluid (CSF). Using a technique involving solvent extraction-PAAC purification, this acid was retracted with N,N-Bis (trimethylsilyl) acetamide (BSTFA) (Madubuike, U., M.Sc. Thesis, UHS Sch. of Grad. & Postdoc. Studies) (1975) and the derivatives estmated by GLC. Radioactive PAAC was added to the initial samples and carriers without the presence of PAAc in a uniform internal standard for recovery (PAAC recovery range, as PAAC-BSA, 17-34%). This method provided a detection limit of about 2 ng of PAAC and allowed differentiation of PAAC and other possible interfering binogenic acids, e.g., mandelic, homovanillic, benzoic, m-naphthyl PAAC and 3,4-dihydroxy PAAC, RT of 13.3, 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 sample, approximately 10 ml fluid, was obtained by pooling several (3-5) individual samples. Measure-

ment of PAAC could be of importance as one of the parameters used in elucidating the postulated role of PAAC in a number of disease conditions, including hyperplakennia and affective and extra-

pyramidal disorders. Supported in part by NIH General Research Support Grant FR-3566 and by Unv. of Health Sciences/Sch. of Grad. & Postdoc. Studies.

REGIONAL CEREBRAL GLUCOSE METABOLISM DURING HYPOXIA. William A.

Phenotypic 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 re-

 mains unclear. We assessed qualitative differences in regional glucose metabolism by the 2-3-deoxy-D-glucose (2-3-DOG) technique under conditions of adequate hypoxia in the physiologically-controlled "Levine" rat. Male rats weighing 250-300 gm were paralyzed and ventilated with 70% N20-30% O2. The right common carotid artery was ligated and the Fio2 of the inspired gas lowered to produce an arterial Po2 of 28-32 mm Hg; arterial blood pressure was maintained above 100 mm Hg. After 10 min of equilibration, 50 μCi 13C-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 ligat-

ed) showed no right-left asymmetries and gray matter was darker than white matter. Autoradiograms of hypoxic rats reveal-

ed 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 stratum and hippocampus. Hypoxia induced bilateral increases in 2-3-DOG metabolism in both hemispheres; the cortex of the right hemisphere was more intense than on the left. The cortex of the hypoxic rat contained less energy reserves than regions of normal oxygen tension. The hypoxia-induced changes in regional 2-DOG phosphorylation may reflect an increased energy demand and/or an increase in energy glycolysis. If anerobic glycolysis were increased only to meet normal energy demands, one would expect an identical increase 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.

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 Cu(II)-L-DOPA and a number of metal-Cu(II)-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 brain and circulating blood after i.v. administration of Cu(II)-chelate remained steady beyond 90 minutes. Zn(II)-L-DOPA-ATP (1:1:1) and the combination drug, pools of rat brain, studies were undertaken on the aminoacids effected by the combination drug. Whereas the brain levels of the aminoacids aected by the combination drug dropped drastically beyond 30 minutes, those of the Cu(II)-chelate remained steady beyond 90 minutes. Detailed results on 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.

BRAIN RESISTANCE TO PROTEIN LOSS ON RESTRICTED PROTEIN INTAKE IN WEANLING RATS. Richard R. Rebert, Robert L. Chronister, Herbert K. Longenecker and L. Preston Morgan* 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%, 5%, 10%, 15%, 20%, 24%, 28% and Purina Rat Chow of 24% or "normal" dietary protein intake. Feeding and drinking were ad lib. with each animal in its own cag. Body weights, food and water intake were measured daily. 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%, 5%, 10%, 15%, 20%, 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 weaning 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 restrictions is emphasized. This study was supported by an Intramural Grant (#22610) from the University of South Alabama.

Five day cultures of neuroblastoma, human hybrid of neuroblastoma cells (NIM-N) and glioma cells (NIM-D) were grown in cell culture and protein species were separated and identified in the first dimension initially utilizing isoelectric focusing and proteins were separated in the second dimension by SDS acrylamide gels. Proteins were identified which were dominantly expressed in neuroblastoma cells and also in hybrid cells and proteins were also identified dominantly expressed in glioma and also hybrid cell cultures. Specific protein species were identified which were significantly expressed in neuroblastoma cells and much reduced in glioma cells, and also conversely. The hybrid cell line expressed many of the neuroblastoma type proteins and relatively few of the glioma type proteins. Properties labelled as G, G and QS 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 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 Ajum mouse brain. These studies emphasize the point that the determination of an enzyme activity or protein concentration in the same sample of brain is in actuality, the summed expression of both neuron and glioma monitored and regulating the genetic expression of the opposite cell. Biochemical neuronal-glia interaction is in operation in the intact brain as suggested. The finding of the z protein in Ajum mouse brain and may be a central mechanism of differentiation within the mammalian brain.

Partially supported by a Grant of J.M.Vargas.
ACETYLCHOLINE AND SEROTONIN CONTENT IN BRAIN AREAS OF RATS DURING PERIOD OF BEHAVIORAL DEPRESSION FOLLOWING D,L-5-HYDROXYTRYPTOPHAN ADMINISTRATION. P.A. Shen, J.R. Hingtgen* and M.H. Aprison, Depts. of Psychiatry and Biochem. and Institute of Psychiatric Research, Indiana U. School of Medicine, Indianapolis, IN 46202.

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. 44: 144, 1971): hippocampus, striatum (ST), telencephalon, 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 ST 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).

MEMBRANE FLUIDITY AND SYNTAPOSOMAL ATPASES. Albert Y. Sun, Sinclair Comparative Medicine Research Farm and Department of Biochemistry, University of Missouri, Columbia, MO 65201.

Several experimental data have indicated the dependency of synaptic membrane ATPases (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 responses to various concentrations of ethanol was observed, as amnonium chloride precipitates ATPase with limited (Na+K+)-ATPase. The enzyme required Na+ concentration (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 USPHS Grant AA02054).


The turnover rates of the putative neurotransmitters in various brain regions can 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) and epinephrine (Epi) was measured in the cerebral cortex and striatum after injections of radioligands. Rats were injected with 0.5 ml 3H-acetylcholine, 1.0 ml 3H-tyrosine, 0.5 ml 3H-Choline and 0.2 ml 14C-glucose through chronic jugular catheters. Five rats were sacrificed by near freezing in liquid nitrogen at 60 min after the administration of 3H-acetylcholine, 3H-tyrosine and 14C-glucose and 7 min after 3H-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 IN 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 turnover rates of the putative neurotransmitters as well as low molecular weight phosphate esters. In this study, the effect of the phosphatase on brain ACh was assayed for content and radioactivity by a radioenzymatic method and paper electrophoresis, followed by a recently described procedure (Shea and Aprison, ANAL. BIOCHEM. 56, 165, 1973) from another portion of the FA/A extract. The amino acid portion of the neurotransmitter fractions was then extracted into 5% TCA from a 10% portion of the powdered tissue samples and their respective dihydroxyphenyl derivatives separated by two dimensional TLC (Brenner et al., Exp. Cell Res. 17, 149, 1963) and assayed for radioactivity. DA turnover in the Str (197.0 pmols/mg protein-hr) was ten times that seen in the CC (18.9 pmols/mg protein-hr); and NE turnover was four times higher in the CC than in the Str (26.9 and 8.9 pmols/mg protein-hr respectively). Asp and Glu turnover in the CC was twice that in the Str (Asp 40.8 and 23.8 pmols/mg protein-hr; Glu 159.4 and 87.6 pmols/mg protein-hr). Glu turnover in the Str (24.5 pmols/mg protein-hr) was three times that in the CC (9.6 pmols/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 NIMH Grant DA-01999-02.)

INACTIVATION OF BRAIN GLUTAMATE DECARBOXYLASE BY ALKALINE PHOSPHATASE. P.T. Spe, 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 enzymes, as amnonium chloride precipitates APase activity by PLP; and (2) the inactivation of GAD leads to a sharp decrease in the energy of activation. From Lineweaver-Burk plots, the inactivation of GAD leads to a sharp decrease in the energy of activation. The inactivation follows second order kinetics, with a half-life estimated as 30 min. at the concentration of AP used following data: (1) The inactivated GAD cannot be reversed to active GAD by any phosphate or from various brain regions. After the AP activity was completely inhibited by 0.2 M phosphate, GAD activity was determined directly in the incubation mixture measured radiometrically from 1-14C-l-glutamate. Alternatively, GAD activity was also measured in the cerebral cortex and striatum after injections of 3H-glutamate, 3H-tyrosine and 14C-glucose through chronic jugular catheters. Five rats were sacrificed by near freezing in liquid nitrogen at 60 min after the administration of 3H-glutamate, 3H-tyrosine and 14C-glucose and 7 min after 3H-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 IN 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 turnover rates of the putative neurotransmitters. Asp and Glu turnover in the CC was twice that in the Str (Asp 40.8 and 23.8 pmols/mg protein-hr; Glu 159.4 and 87.6 pmols/mg protein-hr). Glu turnover in the Str (24.5 pmols/mg protein-hr) was three times that in the CC (9.6 pmols/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 NIMH Grant DA-01999-02.)

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 mg/ml). The ability of AP to inactivate GAD, like its ability to dephosphorylate p-nitrophophenyl 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 Vmax, 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 cystolys 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) AP cannot be prevented by the addition of PLP at high concentrations. The other possibility is that AP may act by hydrolyzing protein-bound PLP, leading to inactivation of GAD. Both possibilities are being tested. (Supported in part by MH-29237)
1040 EFFECTS OF SODIUM OCTANOATE ON BLOOD-BRAIN BARRIER TRANSPORT.

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 studies, 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 mW/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 experiment, rats were decapitated in 5 seconds, the brains quickly removed, and sections prepared for scintillation counting. The data will be compared with that of rabbit skeletal muscle myosin. Supported in part by NIH Grant NS 11824, The Clinical Center for Research in Parkinson's and Allied Diseases, NICHD Grant NS 11631.

1041 EFFECTS OF CATECHOLAMINE METABOLITES AND THEIR OXIDATIVE PRODUCTS ON THE UPTAKE OF 3H-NOREPINEPHRINE 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 (Sachse & Jonsson, C. (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 synaptosomal fraction. The forebrains of adult female Sprague-Dawley rats were used to isolate a crude synaptosomal fraction which was then resuspended in 0.2 M solution. A portion of this suspension was mixed with a physiological salt solution containing 25 mTET (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 (Acid). The corresponding quinone was added to NEP for each experiment was 10 min. Immediately following each experiment, standard procedures were employed to measure the uptake of 3H-NE into the synaptosomes found in NEP. The effects of the oxidation products of CAMT varied from essentially complete inhibition at 10-5 M to almost no inhibition at 10-2 M. There was a corresponding CAMT inhibited condition that is higher at most concentrations. At the higher concentrations studied, the electrochemical data indicate substantial secondary oxidation reactions occur in the presence of synaptosomes.

This research was supported by the Easter Seal Research Foundation, Grant #W-7014, National Easter Seal Society.


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 trypsinic 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 1X8). 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 HIO

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.
NEUROCYTOLOGY
Apical blebbing of the choroid plexus has been observed in 8 species. One component of this phenomenon which included am­
phibian, reptilian and mammalian forms. A secretory function for
the apical protrusions was suggested by ultrastructurally observ­
ed stages including attached and free-floating profiles which
underwent swelling and eventual rupture in the CSF space. Mem­
brane dynamics studies using ruthenium red and alcin blue mark­
er the observed blebbing and formation and secretion of pro­
tions of choroidal epithelium and the stimulation of bleb forma­
tion by paracetamol speak against the artefactual origin of
the blebs.

Protein synthesis studies utilising intravenously administered
C14-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 autoradi­
ographs following H3-leucine and H3-phenylalanine administration
have demonstrated preferential intracellular labelling in cho­
roidal epithelium as well as blebs, both in vivo and in tissue
culture preparations. These findings, together with previous re­
ports, suggest that the apical blebbing phenomenon represents a
physiologically significant source of CSF proteins.

Supported by U. S. Public Health Grant # R01 NS12906-01Al.

In vitro experiments with a series of amino acids have shown
that some were incorporated more efficiently than others. For
instance, histidine and lysine were incorporated at a rate of 1.8
μC/mg protein/hr, compared to 0.2 μC/mg protein/hr for
leucine. These results demonstrate specific population differences
on post-synaptic structures in the SPN and suggest that gastroin­
testinal peptides are essential for studies of synaptic reorganiza­tion.

In the developing CNS, neuronal processes grow to target
neurons and form synaptic contacts. The precise mechanism 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 the forma­
tion of synaptic contacts. We have investigated the effect of
modifying surface charges of developing neurons. During synapto­
genesis, compounds with different charges were added to cell
cultures of the CNS.

In the developing CNS, neuronal processes grow to target
neurons and form synaptic contacts. The precise mechanism 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 the forma­
tion of synaptic contacts. We have investigated the effect of
modifying surface charges of developing neurons. During synapto­
genesis, compounds with different charges were added to cell
cultures of the CNS.

Additionally, extensive staining was observed in thalamus, hippocampus, caudate, cerebral cortex, septum, and non-neuro­
secretory hypothalamic areas. A developing approach was made
for impregnating neurons of various types and locations. How­
ever, the staining observed in thalamus and hypothalamus is selective, as some areas (e.g., ventromedial hypothalamic nu­
cleus) stain while others (e.g., suprachiasmatic nucleus) do not. Whether this selectivity is a result of specificity of puromycin or if
its particular interaction with certain neurons has not been deter­
mined.

Supported by research grant no. NS05140 from NIMH.

APPARENT PRESYNAPTIC ELEMENTS FORMED ON POLY-BASIC COATED BEADS.
Richard W. Burry and John G. Wood, Department of Anatomy, Uni­
versity 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 mechanism 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 the forma­
tion of synaptic contacts. We have investigated the effect of
modifying surface charges of developing neurons. During synapto­
genesis, compounds with different charges were added to cell
cultures of the CNS.

Dispersed cell cultures of 2 day old rat cerebelums were pre­
pared as described by Lee (Brain Res., 69 (1974) 75). In order to
stabilize the compounds added to the living cultures, proteins of
different charge were coated on to cyamogen bromide
activated sarcohesive 48 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 (poly­
glutamate) did not attach. Beads coated with Con A also at¬
tached to the cultures apparently via a sugar binding mechanism and not
by the electrostatic mechanism of the poly-basic compounds.

Within 24 hrs, Con A coated beads were observed to have
grown up onto the attached beads. Electron microscopic observations
showed swellings of neuronal processes which were closely applied
to the poly-basic coated bead. Concentration of 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 are formed by 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 also
to the poly-basic proteins 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 may contribute some of the 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 (G.M.)

DIGITIZER ASSISTED QUANTITATIVE STUDY OF BOUTONS CONTACTING
NEURONS IN THE SACRAL PARASYMPATHETIC NUCLEUS. H. Keith Brown
and Ronnevi, J. Neurocytol. 6:195-210). The purpose of this
study is to describe the frequency of occurrence and postsynaptic
coverage of each type of bouton found in the spinal nucleus re­

lected by paracentesis speak against the artefactual origin of
the blebs.

Protein synthesis studies utilising intravenously administered
C14-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 autoradi­
ographs following H3-leucine and H3-phenylalanine administration
have demonstrated preferential intracellular labelling in cho­
roidal epithelium as well as blebs, both in vivo and in tissue
culture preparations. These findings, together with previous re­
ports, suggest that the apical blebbing phenomenon represents a
physiologically significant source of CSF proteins.

Supported by U. S. Public Health Grant # R01 NS12906-01Al.

In vitro experiments with a series of amino acids have shown
that some were incorporated more efficiently than others. For
instance, histidine and lysine were incorporated at a rate of 1.8
μC/mg protein/hr, compared to 0.2 μC/mg protein/hr for
leucine. These results demonstrate specific population differences
on post-synaptic structures in the SPN and suggest that gastroin­
testinal peptides are essential for studies of synaptic reorganiza­tion.

In the developing CNS, neuronal processes grow to target
neurons and form synaptic contacts. The precise mechanism 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 the forma­
tion of synaptic contacts. We have investigated the effect of
modifying surface charges of developing neurons. During synapto­
genesis, compounds with different charges were added to cell
cultures of the CNS.

Dispersed cell cultures of 2 day old rat cerebelums were pre­
pared as described by Lee (Brain Res., 69 (1974) 75). In order to
stabilize the compounds added to the living cultures, proteins of
different charge were coated on to cyamogen bromide
activated sarcohesive 48 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 (poly­
glutamate) did not attach. Beads coated with Con A also at¬
tached to the cultures apparently via a sugar binding mechanism and not
by the electrostatic mechanism of the poly-basic compounds.

Within 24 hrs, Con A coated beads were observed to have
grown up onto the attached beads. Electron microscopic observations
showed swellings of neuronal processes which were closely applied
to the poly-basic coated bead. Concentration of 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 are formed by 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 also
to the poly-basic proteins running over them, but no apparent presynaptic elements were seen adjacent to the Con A coated bead.
NUCLEAR INVAGINATIONS IN DEVELOPING HAMSTER NEURONS. MB. Tank Huschmann and A. LaVelle. Dept. Anat. & Gen. Nursing, Coll. Med. & Coll. Nursing, Univ. Ill. Med. Ctr., Chicago, IL 60612. A high number of nuclear invaginations have been frequently observed in rapidly growing and in adult neurons undergoing the retrograde degeneration. 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 nuclear apparatus is increasing in size; with lagging off of this processivity, in maturity, the invaginations disappear or are greatly diminished (LaVelle & LaVelle; Tennyson, Rev. Neurobiol., Hinrich, et al., 1970). In the large sensory neurons, for invagination has been particularly noted during the peak of chromatolysis (Lieberman, 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., 61, 1943) and their increase may be related to enhanced neuronal metabolism (Lieberman, Rev. Neurobiol. 14; 49, 1971).

In the case of the hamster, in pyramidal cells of layer V, the pattern of very rapid and perikaryal growth begins from newborn to 2 years of age by which time adult nuclear and perikaryal volumes are reached. At about 5 days, nuclear invaginations become apparent and their depth and frequency increased progressively with age in the pyramidal cell (Spearman & Coll. Nursing, Univ. Ill. Med. Ctr., Chicago, IL 60612). It would appear that in these hamster pyramidal cells the persistence of invaginations with age through maturity may indicate a continuum of high metabolic activity which was established during the early growth period. This speculation has yet to be confirmed by more direct metabolic studies.

DEVELOPMENT OF SUPRAEPENDYMAL NEURONS ASSOCIATED WITH THE MEDIOLATERAL EMINENCE OF THE HAMSTER. J.P. Card* 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, 797) on the infundibular recess of the adult hamster a well organized cluster of intraventricular nerve cells and processes was consistently found associated with the nonilluminated ependyma of the median eminence. The following study was undertaken to examine the developmental features of this cluster during the perinatal brain. Brains were collected and fixed with Karnovsky's aldehyde fixative on days of gestation 11-17 and on day 12, 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 autoradiographic processing for scanning electron microscopy (SEM) examination. Selected specimens at each stage of development were subsequently processed for transmission electron microscopy (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. In the adult hamster, the array 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 nuclei of supposedly subcellular, diastotological procedure compatible with high resolution radioautography after administration of [3H]DG was developed. In adult rats, vascular perfusion using mixed products resulted in relatively high radioactivity. Independent supraependymal cells and processes were first visible on day 12 of gestation, and a well organized neuronal cluster of high metabolic activity was observed as early as day 13 of gestation and was always present by parturition (day 16) and thereafter. The ultrastructural characteristics described above could be related specifically to the adult hamster.

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, School of Medicine, University of Tennessee Center for the Health Sciences, Memphis, TN 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 large 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 with increased irregularly distributed 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 the experimental period 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 discernible, especially in the adult.

In conclusion, there appears to be a loss of synaptic vesicle membrane from cone terminals in long-term dark adaptation when membrane recycling process is probably maximized. This 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 synaptic 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.

ADAPTATION OF THE DEOXYGLUCOSE METHOD FOR USE AT CELLULAR LEVEL. Michel H. Des Rosiers* and Laurent Descarries, Centre de recherches en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada H3C 3TR.

To make the deoxyglucose (DG) method (Sokoloff et al., 1977) available at cellular and possibly subcellular levels, a histological procedure compatible with high resolution radioautography after administration of [3H]DG was developed. In adult rats, vascular perfusion using mixed products resulted in relatively high radioactivity. 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-[3H]2-deoxy-D-glucose, 600 ml of 3.5% glutaraldehyde in 0.05 M phosphate buffer (3-4 min), 500 ml of 1% OsO₄, in 0.1 M phosphate buffer (3-4 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 totum. One-thick sections were prepared for radioautography according to standard dipping techniques. To evaluate the global reactivity of DG and DG-6-phosphate aminophosphomonoesterase activities, specific areas were chosen after examining histological sections in light and electron microscopy. Since DG and DG-6-phosphate are applicable at cellular and possibly subcellular levels, a histological procedure compatible with standard dipping techniques. To evaluate the global reactivity of DG and DG-6-phosphate aminophosphomonoesterase activities, specific areas were chosen after examining histological sections in light and electron microscopy.
Gliaal filaments treated section

Fig. 1, Control (1:1000). GF of Myxicola have different immunologic properties. Preimmunization rabbit serum, specific antiserum absorbed with microtubules and blocks axoplasmic transport (Ghetti and Ochs, Abst. Soc. Neurosci., 1977). The aim of the present communication is to: (1) to describe the neurofibrillary changes in Myxicola and other microtubule-destroying agents (MTTs), (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 tissue of rabbits intracranially injected with 25, 50 and 100 µg of either MTT or MTPP. The retina of rabbits intracranially injected with 3 µg of MTT was studied by electron microscopy 7 days following injection. The results are as follows: (1) MTT induced a severe neurofibrillary degeneration in neurons of the spinal cord, medulla and pons between 45 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 to a variety of stimuli in vivo were also involved: retina, thalamus, hypothalamus, amygdala, midbrain, substantia nigra, hippocampus, and neocortex. All responded with a number of different changes on varying times following injection. (3) The retina and auditory nuclei responded to the shock of the current. (4) The fetus, on the other hand, responded with a number of changes that could not be related to any known effects of the agent of injection. (5) The changes in neurons did not seem to follow any particular pattern, although there was a tendency for changes to be more severe in the midbrain and pons between 48 and 72 hours following injection.

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 an antibody of cytoplasmic actin. The study was performed on unfixed, frozen, freeze-fractured preparations.
A population of cells which rapidly incorporate extracellular material has been identified by finding immediately beneath the ependymal cells of the cerebral ventricles of the toad (Bufo marinus). The ventricular system was perfused with 1H-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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is 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 in 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 similar to cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Observation of HRP uptake by subependymal cells is readily distinguished from other central nervous system cell types.
1063 MORPHOLOGY APPEARED UNREMARKABLE. WITHIN 6H OF BRAIN INJURY, CONTAINING VESICLES. WITHIN THESE SAME GROUPS OF NEURONS, AFTER CELLULAR DEGENERATION WAS NOTED AND SO THE FATE OF THE EVIDENCE OF PHYSICAL DAMAGE AND ALL OTHER ASPECTS OF CELLULAR RELATED TO THE TRAUMATIC INJURY AND WAS NOT SECONDARY TO VARIOUS NUCLEOLAR PEROXIDASE UPTAKE, NO ULTRASTRUCTURAL MANIFESTATION OF NO NEURONAL FLOODING WITH PEROXIDASE WAS OBSERVED. NO EVIDENCE FOR PHYSICAL DAMAGE AND ALL OTHER ASPECTS OF CELLULAR CONSEQUENCES OF LDCV'S IN AXON TERMINALS (F/μ²) 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 PRESYMPATITIC TERMINALS DECREASED SIGNIFICANTLY AFTER A MINIMUM SPREADING AND RECOVERY OF SYNAPTIC DENSITY AND THE CONCENTRATION OF LDCV'S IN AXON TERMINALS RECOVERED TO 47% OF CONTROLS, WHEREAS THE CONCENTRATION OF CLEAR-CORED VESICLES COMPLETELY RECOVERED AN AEBONIES OF MYELIN SHEATHS IN PARANODAL Axonal membranes are specialized for 


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) through its inner or outer edges. A movement of cholesterol from axon into MS was also suggested (Rawlins, F.A. J. Cell Biol. 68: 480, 1976). Such neuronal peroxidase uptake appeared to be directly related to the traumatic injury and was not secondary to various traumatic hemodynamic episodes. Supported by NIH Grant NS-12352.

Supported by NIH Grant NS-55297-02/52

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 with Nissl and Holmes methods. Several changes in the arrangement of Nissl substance and the argyrophilia of the neurons were observed. These changes occurred very rapidly, as early as 12 hrs postoperatively in the animals with bulb lesions and at 48 hrs for animals with LOT lesions. Delta-coned retrograde degeneration neurons occur 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 bulb lesion.

The mitral cells caudal to the direct bulb 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 deafferentation 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. Grazier and by NIH training grant ST32NS 07010.


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 apical dendrites of lumbar motoneurons 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 venticular in 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 area of rostral lumbar segments, was the appearance of pinocytotic enclones (3-15 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 thickenets (see Stenness and Stenness, Brain Res. 31: 67, 1971) of the ipsilateral gray matter, where they closely opposed other HRP-positive processes, (3) otherwise non-reactive dendritic thickenets of the contralateral gray matter and (4) close apposition to neurons whose dendrite 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 opposed to dendrites and neuronal perikarya of the other side. (Supported by USPHS grant NS 12211)
NEURO-ENDOCRINOLOGY
pineal influence on adrenocorticotropic hormone (ACTH)

1070

1071


Stimulation of the vagina and cervix by the intramissions of the male rat during copulation is required to initiate the neuroendocrine events (progestational state) necessary for successful pregnancy (Dullar, J. Comp. Physiol. Psychol. 69:613, 1969). In the present investigation, we used the (14C)-deoxyglucose (2DG) method (Kennedy, DesRosiers, Jehle, Reichlin, Sharpe & Sokoloff, Science 172:650, 1971) to identify brain areas which increased functional activity in response to vaginococital stimulation.

Unanesthetized, behaviorally receptive female rats were gently restrained and stimulated with a smooth metal rod attached to a vibratory engraving tool. The vaginococital 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 intracar dially via a chronically implanted jugular catheter. There were two groups of control animals: (1) females receiving no vaginococital 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 vaginococital stimulation (Carrer & Taleisnik, Endocrinology 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.)

sexual differences in pattern of hormone accumulation in the brain of a songbird. Arthur P. Arnold and Albert Salit
dep. 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 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 reflect 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 detected sexual differences in distribution of hormone-accumulating cells. The following areas contain labelled cells: both striae terminalis 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.

Effects of castration and estrus cycle state on monoamine-stimulated adenylate cyclase activity in rat hypothalamus and amygdala. G. A. Barr, H. S. Ann, J. L. Gibbons, and M. H. Maxman. Deps. of Psychi, 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 increases 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.

Adrenaline 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 isopropyl-norepinephrine (100 μM) only during proestrus, and not during other stages. Furthermore, in the anterior hypothalamus, only GPP(NH)p stimulated AC. Amygdaloid AC was stimulated by NE, but not 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 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 that are either primary or secondary to alterations in presynaptic activity.

(Supported by Grants MH 06418, NS 09649, and RR 05597)
EFFECT OF ANTERO-VENTRAL THIRD VENTRICLE LESIONS ON ANTIDIURETIC RESPONSES TO CENTRAL ANGIOTENSIN II. Steven L. Bealer, M. Ian Phillips and Philip Schmidt. Depts. Psychology, Physiology and Internal Med., Univ. Iowa, Iowa City, IA 52242. Electroclystic lesions of periventricular tissue of the antero-ventral third ventricle (AV3V) typically render rats aphagic 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, R12-R129). The continued diuresis during lesions indicates that antidiuretic hormone (ADH) mechanisms of fluid conservation have been compromised by AV3V ablation. The present experiment was designed to determine if AV3 lesions attenuate ADH release in response to intraventricular (IVT) injections of angiotensin II (AI1), hypertonic NaCl, and phenylephrine during the adipsic period. Following electrolytic ablation of the AV3 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 the blood and urine parameters were recorded following IVT injections of 100 ng and 500 ng AI1, 1 ul 3% NaCl, and 50 ug phenylephrine. In the AV3 lesioned rats, increases in blood pressure and urine conductance, as well as the decreased urine flow rate normally evoked by AI1 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 if ADH release in response to intraventricular (IVT) injections of Angiotensin II (AI1), hypertonic NaCl, and phenylephrine during the adipsic period.

ALTERED NEUROANATOMICAL ORGANIZATION IN THE CENTRAL NERVOUS SYSTEM OF THE GENETICALLY OBSESE (db/db) MOUSE. David R. Berestet and Bernard Schambach. Laboratoires de Recherches Medicales, Geneva, SWITZERLAND. The genetically obese (db/db) mouse is characterized by exaggerated body weight gain, hyperphagia and hyperinsulinemia. These and other endocrine related abnormalities have suggested a geneticized central nervous system (CNS) disorder, possibly hypothalamic. These results have been obtained from nine week old male genetically obese (C57BL/6J db/db) mice and lean (C57BL/6J) control mice: (1) db/db mice have greatly reduced total brain wet weight (-14.6%) that is not due to altered water retention. (2) Using a planimetric technique, db/db neurons demonstrate significantly reduced soma cross-sectional areas in the ventromedial hypothalamic nucleus. (3) Only lateral hypothalamic area neurons in db/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 db/db 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.)

CORTICOSTERONE INDUCED PROTEINS IN THE PITUITARY OF ADRENALECTOMIZED MALE RATS. Margery C. Beinfeld* and Paul M. Feckman*. (SPON: R. A. Cohen. Dept. Psychol. and Sch. of Med., 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 [3H] labeled leucine while controls were incubated with [3H] 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.

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 include the soma by axonal transport, and in each cell these peptides account for 40-50% of the total incorporation of [3H]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 [3H]leucine into total protein, and can thus induce a doubling of the somatic content of these peptides. However, the extent of incorporation of [3H]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 EFFECT ON SEXUAL RECEPTIVITY. Jeffrey D. Blaustein, Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

The synthetic progestin, 17β-21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R 5020), which binds with higher affinity and dissociates less rapidly than progesterone, 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 progesterone-specific. 100X excess of radiolabeled progesterone, 9α-dihydroprogesterone, or progesterone sulfate inhibited 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, but 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 (148%). 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 progesterin receptor content increases between 14-24 hr, peaks by 40-64 hr and returns to 88 hr. This time course correlates well with the previously reported rapid increase in serum LH in response to estradiol benzoate in ovariectomized guinea pigs. Following termination of heat, a refractory period is seen during which time an additional injection of progesterone does not facilitate lordosis again. The failure of progesterone to facilitate lordosis correlares 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.


Lesions of rostral septum lead to increased serum GH and somatic growth and to decreased pituitary GH content and concentration in adult hamsters (Boer et al., Neuropeptides, 1977, 23, 133). We examined the hypothesis that this effect was due to damage to fibers of passage by producing bilateral lesions of the lateral septum in 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 growth was significantly greater (p < .01) 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 pretrial state (9.6 ± 1.9 vs 21.1 ± 0.2 μg SHAP/mL, p < .01) and serum insulin was increased in animals killed on day 14 (14.2 ± 1.7 vs 1.3 ng/mL, p < .01). HIPPO hamsters were significantly fatter than CON (17.9 ± 1.1% vs 11.0% p < .01) with obesity accounting for 25% of weight differences between the groups. HIPPO hamsters had significant depletion of serotonin (SERT), p < .05, and NE p < .01 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 serotonin, NE and/or other nerve fibers traveling through rostral septum to HIP inhibit GH secretion and somatic growth in adult hamsters.


Dopamine (DA) was measured in the pituitary anterior lobe and median eminence from lactating rats using a modification of Cowen and Lennarz 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 case of suckling 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 findings suggest a role for adrenocorticotropic DA in the regulation of prolactin release.

1081 CHARACTERISTICS OF THE ARCULATE NEURONS IN RESPONSE TO MEDIAL PREOPTIC STIMULATION. Carlos N. Contreras* and El 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 approach 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 at the same stage. 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 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 16.4% (9/59, 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 over 30 msec; 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 were polysynaptic neurons. Distribution of neurons 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) MP0 stimulation induced more activation in the antidepressively identified neurons than polysynaptic neurons, and MP0 stimulation induced more depression in the polysynaptic neurons than antidepressively identified neurons in both OVX and EB groups. 5) The MP0 activity was slightly higher in the EB groups than in the OVX groups. 6) MP0 stimulation of lower percentages in the EB groups and lower percentages of depressed neurons were recorded in the EB group. 7) EB treatment induced prolonged MP0 activation and increased MP0 depression in the antidepressively identified and the polysynaptic neurons, while EB treatment produced shortened MP0 activation in the polysynaptic neurons.

It is concluded that 1) a major portion of antidepressively identified and polysynaptic neurons originate from the arcuate nucleus; 2) those neurons were activated rather than depressed by MP0 stimulation; and 3) this facilitation 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 unrestricted access to food. 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 in each BW condition were given daily subcutaneous injections of 3.0 g/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 treated group were 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.

1(Present Address: Neuropsych, Br., DME, DMRAR-CL-MN, AFSC, MD 21010)


Intravenously administered 125I-labeled iodothyronines (T3, T4) reaching the rat brain become highly localized in synaptosomes (SYN). Although both hormones are taken up to the same degree in brain synaptosomes, T3 is approximately 2-fold more concentrated than T4 within nerve terminals. Measurement of endogenous hormone concentrations in SYN (isotope equilibrium studies) confirm significant enrichment in T3 relative to T4. 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 iodothyroxine transport into SYN, enriched nerve ending preparations, separated from other particulate fractions (as discontinuous sucrose density gradients), were incubated under 95% O2-5% CO2 in bicarbonate buffered salt solution (artificial CSF, Glowinski) containing 1x10−5M T3 or T4. To verify uptake into, as distinguished from binding to, SYN vesicles, portions of prelabeled SYN were treated with either CF3 or H2O; 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, with rates measured during the first 3 min (0.2–0.3 pmol/min/mg SYN), reached a plateau in 10 min and were similar for both T3 and T4* throughout the 30 min period of observation. At 30 min, iodothyronine* was 40–50 fold more concentrated than T4 in SYN than in medium. Addition of increasing concentrations of unlabeled hormone, over a range of 1x10−7 to 1x10−6M, 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 T3 and T4 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 iodothyroxine uptake thus far measured: high initial velocity, and low amino acid competition, suggest that neuronal uptake, and membrane 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.


Melanophore stimulating hormone (MSH) is known to effect melanin dispersion in amphibians and reptiles, but has little effect on melanin pigment in other species. It is concluded that stimulation of the pineal region by light or electrical current promotes the release of MSH from the pineal gland into the bloodstream, which is then carried to the skin where it produces a change in coloration. It has long been reported that the sensory input controlling MSH secretion is from photic cues processed through the retina and the retina controls MSH secretion via hypothalamic neurons either through a humoral step or by direct neuronal connections to the pars intermedia with a resulting spontaneous secretion of MSH. We have been able to induce darkening of Rana pipiens melanophores in vivo by electrically stimulating the surface of the diencephalon 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 affect skin darkening. Continuous electrical stimulation of the hypothalamic region elicited reciprocal darkening and lightening of the pineal complex and the skin of the animal. We propose that the pineal complex is the light receptor which regulates MSH release via neural tracts through the hypothalamus which innervate pars intermedia secretory cells.

Supported by funds from the Medical Research Service, Veterans Administration Hospital, Philadelphia, Pa.

The ventromedial nucleus (VMN) of the hypothalamus has been implicated consistently in the control of female 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 whether lesions are recovered from the half of the hypothalamus containing the VMN, and whether lesions were recovered from the contralateral side of the hypothalamus, or whether lesions were conducted. Seven of the 12 females exhibited lordotic behavior on at least one of the tests (tests 1-3, 6, 8 of 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 (as % of hypothalamic levels). Determinations of the radioactivity remaining in the cannula 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. The hypothalamic levels of estradiol (3H-E, 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 revealed a pattern of distribution of radioactivity essentially identical to that shown in whole tissue (individual hypothalamus: 17% of total radioactivity; other regions: < 5% 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.)


Progestosterone inhibits a number of central effects of estrogen and androgen in adult rodents. This inhibitory action may be an important component in the ability of progestosterone to coordinate sexual receptivity and responsiveness to estrous and the ability of estrogen to inhibit the behavior in the absence of ovulation. Another possible role of progestosterone could be to protect the female fetus or neonate from androgenic stimulation during sexual differentiation. The present study examined the effect of progestosterone (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, 50ug 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 decortical effects of TP or EB. 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 females: hypothyroid females: hypothyroid males.

Although P was found to be capable of inhibiting defeminization induced by either EB or TP, this required large doses of P. Even the higher doses failed to reduce sexual functions in the presence of EB or TP. The effective P treatments did result in higher pup mortality and adult gonadal abnormalities. This weak behavioral action of P in combination with EB or TP did 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 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.


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 nucleus neurons. 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 that MSG can be injected into the uterine cervix 16-24 hr before treatment with uterine urethane. Under urethane anesthesia, PRL levels during the nocturnal surge (0130-0430 hr) were 11.8±2.4 ng/ml vs 2.9±0.8 (n=3) 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±1.6 ng/ml in unstimulated rats (n=6).

There is now strong evidence that dopamine (DA) is a physiologically significant inhibitory factor. The purpose of this study was to measure DA levels in hypophysial stalk blood during the PRL surges in uterine anesthetized rats. These long-term ovariectomised rats and last for 6 days. We found in the present study that uterine anesthe-sia (1.1 g/kg SM) did not abate the PRL surge induced by cervical stimulation. Uterine urethane anesthesia, PRL levels during the nocturnal surge (0130-0430 hr) were 11.8±2.4 ng/ml vs 2.9±0.8 (n=3) 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±1.6 ng/ml in unstimulated rats (n=6).
1090 HETEROGENEOUS AXOPLASMIC RETICULUM (AR) IN PEPTIDERGIC 
NEUROSECRETORY (NS) NEURONS: POSSIBLE ROLE IN HORMONE TRANSPORT 
AND VESICLE FORMATION. H. Bigger Dellmann*, Mona Cassell* 
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 hypothalano-neuropophysial 
system (HNS) in several rodent species and in the frog reveal 
marked AR extending from the NS perikarya into the axon 
terminals. This reticulum consists of anastomosing tubes often 
containing electron-dense material similar to that in the neuro-
secretory granulated vesicles (NGV). Constrictions and dilata-
tions are seen along the tubes from which NGV may bud. NGV 
bearing protrusions, like vesicles of reticulum, are apparent 
and reticular connections may exist between NGV (Castel, M. :  Gen.

The AR of the HNS is heterogeneous. In addition to that de-
scribed above, electron dense tubes of AR are seen, often 
fragmenting into vesicles. Furthermore, a type of narrow and 
extensively anastomosing 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 antegrade and retrograde axonal transport of substances, 
other than hormone.

The electron dense AR with which the NGV are associated may 
represent the avenue of fast axonal transport postulated by 
Bigger (In: The Nervous System, D. R. Orme-Johnson, ed. 311-
127, Raven Press, New York, 1975). In the stressed HNS and in 
the fetal and neonatal HNS it becomes abundant, demonstrating 
a remarkable activation of the neuron to the organism's increased 
hormonal demand.

Supported by NIH grant 1 ROI NS14062 (HDD) and in part (MC) by 
BRSF grant 200.

1092 FEMALE FROG REPRODUCTIVE BEHAVIOR ELICITED IN THE 
ABSENCE OF THE OVARIAN. Carol Diekow, Joshua B. 
Wilcox and Richard Wollmann* BIRLOGY DEPARTMENT, 
Adelphi University, Garden City, N.Y. 11530.

A release call is emitted by unreceptive female 
Above when clasped around the trunk; this 
A release call is emitted in receptive females. A recent 
study indicates that water accumulation induced by 
arginine-8 vasotocin causes a chemical dis-
tension that inhibits the call (Diekow, Sci. 199: 
1456, 1975). This finding, that a posterior pit-
uitary hormone is inhibited in unreceptive females, 
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, ovariecetomized female Rana 
pipiens given somatostatin estradiol and/or prog-
esterone 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 ovariecetomized Rana pippens with intraperitoneal 
fluid. These results are consistent with the inter-
pregnantation theory. The release call is inhibited by a 
mechanism that involves water accumulation, but not 
oviduction.

1093 CONTROL OF RELEASE OF ACTH AND VASOPRESSIN BY SUPRAOPTIC AND PARA-
VENTRICULAR NUCLEI. Anne Dorrhorst,* Drew E. Carlson*, 
Said M. Selj*, Alan G. Robinson*, Earl A. Zimmerman and Donald S. Gann. 
Pittsburgh Sch. Med., Pittsburgh, PA 15261 and the College of 

to determine the relative roles of the supraoptic (SON) and of 
the hypothalamic paraventricular (PVN) nuclei in the control of 
release of vasopressin (VP) and of ACTH, we examined the hormonal 
responses to electrical stimulation of these regions. Cats (38) 
were anesthetized with chloralose-urethane, artificially respirated, 
and immobilized with halothane. Electrical stimulation (0.2msec, 
100 Hz, 20sec) were delivered with bipolar coaxial electrodes, 
potential stereotaxically. Blood samples were taken 30 seconds prior to stimulation and 1.5 minutes poststimulation.

Hypothalamus was prevented by simultaneous infusion of equal 
primes of warm isoc respiratory. ACTH and vasopressin were 
measured in plasma by radioimmunoassay. Stimulation sites were 
determined histologically. Eleven brains were immobilized 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 (2.15±0.50 
ng/ml, N=14, P<0.01) and decreased ACTH (2674 pg/ml, N=13, P<0.01). In contrast, stimulation of the hypothalamic paraventricular 
(PVN) nucleus increased vasopressin (2.25±0.50 ng/ml, N=10) 
and decreased ACTH (670 pg/ml, N=10). Stimulation of either nucleus decreased the vasopressin content of neurons 
adjacent to the electrode (30.5±3.9, N=8, P<0.001). In addition, 
the immunostaining of the descending projections from the 
meatal became more prominent. Stimulation in regions which led to 
no hormonal changes yielded no cytochemical differences. Previous 
work has shown that vasopressin neurons of PVN, but not of 
the zona externa of the median eminence. Others have 
reported that retrograde flow of blood from the neural lobe to 
the median eminence results in lobe release of ACTH and 
vasopressin to influence the release of ACTH. The present results 
indicate that both SON and PVN control the release of vasopressin. However, PVN facilitation, release of ACTH and decreased 
vasopressin is not important in the short-term control of ACTH.
EFFECT OF OVARIAN HORMONES ON NEURONAL MEMBRANE SENSITIVITY TO HYPOTHALAMIC-REleasing HORMONES - CAROL A. DALLY AND ROBERT L. MINKER. DEPARTMENT 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 (LHRH) and LHRH analogs was evaluated in ovariectomized (OVX) non-primed and OVX, estrone (E)-progesterone (P) primed female rats. In the OVX rat, the majority of cells recorded were found to be unresponsive to the microelectrophoresis of the releasing hormones, the predominant response being spontaneous activity in non-primed females. In contrast to the relative inability of these neurons to effectuate changes in spontaneous activity in non-primed females, the predominant response in E-P-primed females was excitation. A 2 x 3 x 3 chi square analysis conducted on responses to LHRH 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 LHRH drug with excitation, regardless of the LHRH drug tested. Although the excitatory effects produced by LHRH° are perplexing, these neurons was monitored to detect changes resulting from agonist analog (D-Trp6, Pro9 NH2[LRH+]), and/or LHRH inactive analog (des-Pro9-Gly10[LHRH°]). One hundred and seventy MPO units 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.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Microelectrophoresed</th>
<th>Response in Unprimed Ovariectomized Female Rat</th>
<th>Response in Estradiol-Progesterone Primed Female Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHRH</td>
<td>170</td>
<td>52 (31%)</td>
<td>22 (12%)</td>
</tr>
<tr>
<td>LHRH*</td>
<td>135</td>
<td>46 (34%)</td>
<td>51 (38%)</td>
</tr>
<tr>
<td>LHRH°</td>
<td>96</td>
<td>30 (31%)</td>
<td>14 (15%)</td>
</tr>
</tbody>
</table>

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. The ability of ovarian hormones to affect neuronal membrane sensitivity to LHRH is dependent on the hormonal status of the animal. The postulated role of LHRH in the regulation of reproductive function in adulthood.


Although radioimmunoassay has permitted detection of the neuro-peptides in brain structures, the techniques 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 is characterized by purity and stability of synthetic tripeptide thyrotropin releasing hormone (TRH), the pentapeptide met and leu enkephalin, the decapeptide luteinizing hormone releasing hormone (LHRH), the eleven amino acid peptide substance P and the tetradecapeptide somatotatin. Our equipment consists of a Waters Associates HPLC System with a H-6000 A pump, a USK septumless injector, a reversed phase analytic column and a model 450 variable wavelength U.V. detector. The reversed phase solvent system consisted of acidified saline increasing 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 3 rats injected with histamine increased to 17%± 17 of baseline values while dopamine 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 only to 111% ± 5 (mean ± SE) of baseline values and histamine reduced the number of immunoreactive perikarya visualized and the intensity of stained processes in the male while estrogen enhanced the number of females in the early stages of ovariectomy. These 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 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.


Dopamine secreted by the hypothalamus into the hypophysial stalk vessels has a physiological role, at least tonically, in the inhibition of pituitary prolactin secretion (ENDOCRINOLOGY 102:1985 1976). The role of dopamine in the dynamic regulation of prolactin is still unknown. Putative hypothalamic transmitters which stimulate prolactin secretion when injected into the cerebral ventricles. Histamine antagonists injected with a number of neuropeptides had little effect on prolactin secretion suggesting that the functional site of histamine action is relatively close to the final hypothalamic output to the pituitary. Prolactin levels in 6 rats injected with 50mg ACh into the lateral ventricle caused prolactin to increase to 258± 6 of baseline. This response was not significantly different from the response in rats injected with saline. Prolactin levels in 8 rats injected with ACh probably involve some other hypothalamic factor(s).

POSTNATAL DEVELOPMENT OF THE LUTINIZING HORMONE-REleasing HORMONE (LHRH) SYSTEM IN NORMAL AND MALE TO FEMALE REPRODUCTIVELY ENERGIZED RATS. KAREN E. FELDMANN, JON C. KING, AND ARNOLD A. GERRARD. 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). In the normal group of male and female albino rat pups were randomly assigned to one of 4 experimental treatments on day 2 (day 1 = day of birth): the high dose, the intermediate dose, the control group, and a sham injection group. Each 1000µl of estradiol benzoate (EB) dissolved in 0.05 cc sesame oil, the low dose group with 1 µg EB in 0.05 cc sesame oil, the control group with a volume of oil vehicle only, 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 stained for immunohistochemistry. The distribution of LHRH processes in the neonatal animal resemble that described for the adult: a rostral pathway is located close to the end of the lamina terminalis (WMLT) in the vicinity of the rostral preoptic area (POA) and another caudal one extended throughout the arcuate-neurohypophysial axis (ARC-ME). LHRH positive perikarya were observed 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 seasonal and sexual 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 females than in males at any age. 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 number of females in the early stages of ovariectomy. These 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 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.

NEUROENDOCRINOLOGY
Although no significant sex differences were found in any other portion of relatively large cells (> 12.5 µ) in males than in females; 22.6% were large in males compared to 13.6% in females. The distribution of intracellular structure varies among cell types and among different regions of the brain in males and females, and these differences may reflect differences in functional properties of neurons in these regions.

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 ms at 1/2 amplitude or 4.5 ms at threshold) and followed by afterhyperpolarization. Other cells in vivo responded to current injection with spikes and the mean amplitude of depolarizing current injection could increase the amplitudes of these depolarizations, they probably arise from chemical synaptic relationships (or lack thereof) within relatively circumscribed hypothalamic regions. The paraventricular nucleus (PVN), due to recent anatomical findings of far-reaching and varied cellular interactions, in part because of the difficulty of obtaining intracellular recordings from hypothalamic neurons. Recent work, however, suggests that these results 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 24 hrs of EB priming will provide a time course for the hormone-induced changes.


Little is known about the morphology of intrahypothalamic cell columns,open to the question of whether these results reflect the different functional roles of estrogen in these very dissimilar tissues. When completed, the analysis of samples from the rats receiving 12 and 24 hrs of EB priming will provide a time course for the hormone-induced changes.
were observed in the present study. These data confirm the
been widely used as an index of emotional state. Yet it has been
significantly greater than the basal control group, and significantly
unfamiliarity. The present study extends these findings to the
intensities of psychological stimulation, i.e., degrees of
fore is an ineffective measure of emotionality. This conclusion
recently argued that the adrenal response is insensitive (i.e.,
cannot distinguish various levels of emotional state) and there­
fore is an ineffective measure of emotionality. This conclusion
The present study was carried out to determine whether
These findings of a perisitant rhythm of NAT in vitro
The anatomical locations of estrogen-concentrating cells have
have been described for the major classes of vertebrates (for review,
see Morrell and Pfaff, Am. J. Zool., 1978). In this study
quantitative analyses of the peak areas of hormone concentration
were done to determine the existence of different modes of
in birds and mammals. In rats, the oscilla­
ors to differences in hormone concentration and to investigate possible area­areas 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
347
Quantitative autoradiographic analysis of estrogen concentrating
cells in hypothalamus, preoptic area and amygdala. M. S. Krieger*,

The present study extends these findings to the
rats and examines constant changes in ACTH.
Rats were exposed for 15 min to three apparatus which dif­
ferred 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 signifi­
cantly greater than the basal control group, and significantly
less than any of the experimental groups. Plasma ACTH concen­
trations 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 condi­
tions.

The present study was carried out to determine whether
the rhythm of NAT activity will persist under constant condi­
tions in vitro, thus Leophem children 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,
plasm rinsed and placed into culture. Examination of days
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
occurred during the projected night of day one, remained
low during the projected day period of the intact animal. Media
was changed on day three and then the glands were placed into
3D at which time sampling began at four hour intervals.
A rapid loss of NAT coincided with the beginning of night,
day four. Again the projected night period of day four and fell again during the day period of
day five.

These findings of a persistent rhythm of NAT in vitro
suggest the presence in the avian pineal gland of a
source of endogenous melatonin. Supported by NINDS grant NS-14036 and Research Career Award
RO4 NS 30213 to J.R.P.

The present study was carried out to determine whether
the rhythm of NAT activity will persist under constant condi­
tions in vitro, thus Leophem children were raised from one day
of age under the same lighting regime (12 hours light­12 hours dark). At three weeks of age animals were sacrificed,
plasm rinsed and placed into culture. Examination of days
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
occurred during the projected night of day one, remained
low during the projected day period of the intact animal. Media
was changed on day three and then the glands were placed into
3D at which time sampling began at four hour intervals.
A rapid loss of NAT coincided with the beginning of night,
day four. Again the projected night period of day four and fell again during the day period of
day five.

These findings of a persistent rhythm of NAT in vitro
suggest the presence in the avian pineal gland of a
source of endogenous melatonin. Supported by NINDS grant NS-14036 and Research Career Award
RO4 NS 30213 to J.R.P.


diurnal changes of melatonin in retina, pineal gland, supr­
achiasmatic nucleus, colon, and duodenum of the rat. W. B.
U. Roth, Roch., N. Y. 14042, and Dept. Neurosci., McMaster U.,
Hamilton, Ont., Canada.

Melatonin(M) was localized immunohistologically in a
number of extrapineal areas (Exp. 115:847, 1976; Exp. 33:
66:17, 1977). Although known to exhibit a diurnal fluctuation in
the pigeon, daily cycles of M in other tissues have not been
established. Because of its potential role in physiological
functioning, relative M levels were determined using quantitative
immunohistochemistry in various retinal areas. Tissue samples were harvested at 24 hour
intervals. Male adult Charles River rats, kept on a 12:12 light:dark cycle, were decapitated at 3 hr intervals beginning 3.5 hr after
light onset. LH, P, FSH, and E2 were measured at 6 pm per experiment. Trunk blood
was collected and the pineal gland(P), eyes(6), 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 10μm. 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 CEG photocell having
its maximum spectral sensitivity (515mm) close to the emission
peak of FITC (520mm). The photometer reading between sections
was maintained with anti-M serum and adjacent sections stained
with normal sheep serum were used in all statistical analyses.

Pineal corticosterone exhibited a normal rhythm with peak and troughs at 1:30 pm and 1:30 am respectively, respectively. M content of the P had a single broad peak,
beginning late in the light period and falling sharply to back­
ground level after 7:30 pm. The P showed a similar pattern although only 2 animals were examined at each point. Three tissues exhibited double peaks, or troughs. The C, with intense
fluorescence in the pineal nucleus, exhibited a peak at 7:30 pm
after L-on, with a smaller increase 4:30 pm after L-off. The R,
with fluorescence in both the inner and outer nuclear layers, mirrored the C pattern drop in intensity 7:30 pm after L-on, with a smaller decrease 4:30 pm 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. The R,
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. Possibly influences from these areas of
rhythmic processes and functions in the specific tissues studied

Supported in part by NDRI grant MH-16050.

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 radiomunoassay (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-conjugated melatonin, and have been extensively tested in both in vitro and in vivo systems. One study involved the identification of melatonin 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) L-8-estradiol 2 µg s.c.; 2) R-5020 (a progestational compound) 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 estradiol and neuroleptic-induced tardive dyskinesias, two conditions which involve specific interactions of hormones with the central nervous system, have been found to be resistant to melatonin treatment. The estradiol treatment significantly reduces grooming 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 estradiol and neuroleptic-induced tardive dyskinesias, two conditions which involve specific interactions of hormones with the central nervous system, have been found to be resistant to melatonin treatment. The estradiol treatment significantly reduces grooming 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 estradiol and neuroleptic-induced tardive dyskinesias, two conditions which involve specific interactions of hormones with the central nervous system, have been found to be resistant to melatonin treatment. The estradiol treatment significantly reduces grooming 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 estradiol and neuroleptic-induced tardive dyskinesias, two conditions which involve specific interactions of hormones with the central nervous system, have been found to be resistant to melatonin treatment. The estradiol treatment significantly reduces grooming 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 estradiol and neuroleptic-induced tardive dyskinesias, two conditions which involve specific interactions of hormones with the central nervous system, have been found to be resistant to melatonin treatment. The estradiol treatment significantly reduces grooming and motility. The 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 estradiol and neuroleptic-induced tardive dyskinesias, two conditions which involve specific interactions of hormones with the central nervous system, have been found to be resistant to melatonin treatment. The estradiol treatment significantly reduces grooming and motility.
Seroptin (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: 5HTPT, bufotenine, 5-methoxytryptamine (5MT), bufotenine (B), 5-methoxy-N,N-dimethyltryptamine (5MDT) and 5-methoxytryptophan (5MPW). 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 5MT, B and 5MDT are more potent in stimulating PRL secretion than 5MDT and 5MT. 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 5MDT (5 mg/kg, ip, every 3 hr). Frequent administration of 5MDT, 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 re-determined 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 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 5HT turnover and on receptor sensitivity in vitro, as well as 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 5HT-stimulated PRL secretion. (Supported in part by ADAMHA grants MH 30938 and 29206, RCRA MH 47080 to HYM and USPHS MH 07083 to NS.)

**SECONDARY SYNCHRONIZING STIMULI, THE SUPRACHIASMATIC NUCLEUS (SCN) AND THE ENRAINTMENT OF CIRCADIAN RHYTHMS IN THE RAT.**

E. Y. Moore and B. Siegel*, U. Calif. at San Diego, La Jolla, CA 92039

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 and behavior 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 exhibit 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 divided into 3 groups and given 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 T. SCN lesions abolished the free-running rhythms but reintroduction of a deprivation schedule again produced the regular patterns of activity and drinking but 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.

**THE EFFECTS OF LOMOTROR ACTIVITY ON CEREBELLAR cGMP.**


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 (Lemon et al., submitted), 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, and were 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 exposing the heart to microwave radiation delivered at 2450 megahertz, microwave inactivation system, as modified in our laboratory. Following sacrifice and decapitation, trunk blood was collected for radioimmunoassay for corticosterone (Co) and prolactin (Prl), and brain tissue samples were obtained for radioimmunoassay for Co.

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 were permitted to enter the wheel without microwave irradiation. The Co level was prevented from revolving (1.999 ± 0.103 vs 0.919 ± 0.095 pico­ moles per mg wt.). Both groups had relatively elevated prolactin levels, however, these levels were statistically different between groups suggesting that locomotor activity, not environmental stress, was associated with the elevations in cerebellar cGMP. The data suggest that locomotor activity may be a major contributor to the elevation of cerebellar cGMP in rats exposed to environmental stress.

**EFFECT OF OVARIAN HORMONES ON SYNCHRONY OF HAMSTER CIRCADIAN RHYTHMS.**

Lawrence E. 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 and facing the light source were about 12.2 watts/cm² (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 oscillators peaks in intact animals was 157.5 ± 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 ± 2.5°. Ovariectomized animals entrained to LD 14:10 were given subcutaneous silastic capsules containing estradiol benzoate, progesterone or blanks and significant treatment differences disappeared 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.
1115 EFFECTS OF SUCKLING ON SERUM PROLACTIN LEVELS AND CATECHOLAMINE CONCENTRATIONS AND TURNOVER IN DISCRETE BRAIN REGIONS OF LACTATING RATS. John A. Meyer, Thomas L. O'Donohue*, Lorraine R.-Herrenkohl, Richard R. Gala*, and David M. Jacobowitz. Laboratory of Clinical Science, NIH, Bethesda, MD. 20014

The effects of suckling on serum prolactin (PRL) levels and catecholamine concentrations 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 microdialysis 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.


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 received oestradiol benzoate (EB, 5 μg/day, s.c.). In addition, one group of females received either estradiol benzoate (EB, 5 μg/day, s.c.) or oil, and were killed at 54 hr. In both males and females, EB + P reduced ChAT activity. In the caudal nucleus tractus diagonalis and in the periventricular nucleus and increased ChAT activity in the supraoptic nucleus. EB alone also reduced ChAT activity in the caudal nucleus tractus diagonalis. The present results demonstrate that cholinergic activity in certain discrete brain regions of gonadectomized rats was increased 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.

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, DI-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 a greater number of 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 to a substantial degree of the substantia gelatinosa including aseal segments; they appeared to descend in the dorsolateral funiculus adjacent to the substantia gelatinosa. Lesser numbers of positively stained 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. Use of unabsorbed antiserum to NPS revealed including sacral segments; they appeared to descend in the solitary and dorsal motor vagal nuclei. 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. The authors found 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 substantia gelatinosa of the spinal cord where it may have a role in pain regulation.

Supported by USPHS, NIH Grant AM20337

EFFECTS OF ESTROGEN PRIMING ON SEXUAL BEHAVIOR AND ON STEROID RECEPTORS IN THE FEMALE RAT BRAIN. B. Parsons*, N.J. MacLusky*, M.S. Krueger*, E.S. Utiger, and D. Avila (SPONT: C. Frazmann) Rockefeller University, New York, NY 10021.

Ovariectomized rats pretreated with estradiol benzoate (EB) display greater lordosis and lordosis sensitivity to a subsequent dose of EB than do animals which did not receive pretreatment. This study investigated the effects of 17α-estradiol (E2) pretreatment to normal sexual behavior and to estrogen receptor levels in the rat hypothalamus.

Female rats ovariectomized for 21 days received a 5 mm silastic implant of E2 or of cholesterol (C) for one week. Animals were either sacrificed for chemical analysis, or reimplanted with 5 μg E2 for behavioral testing. Females treated with E2 experienced male 44-46 hr after reimplantation. Then, females received 500 μg progesterone (P), and were retested 4 hr later. Animals pretreated with E2 showed significantly higher lordosis quotients than animals pretreated with C, both when tested with E2 alone and when tested with E2 + P.

Cytosol estrogen receptors were measured by incubating pituitary (P) and hypothalamic, preoptic area and septal region (HPS) extracts pooled from 2 animals with 1 X 10-9M 3H-E2 for 2.5 hr at 4°C. Bound 3H-E2 was measured by gel filtration on Sephadex LH-20. Corrections for non-specific 3H-E2 binding were made using parallel incubations containing 1 X 10-8M unlabeled HPS- and P-estradiol. For progesterone receptor measurements, cytosols were incubated with a synthetic progestogen, 3H-R5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) for 4 hr at 4°C. Bound 3H-R5020 was measured by Sephadex LH-20 gel filtration. Corrections for non-specific binding were made using parallel incubations containing 1 X 10-8M unlabeled R5020, in addition to 0.4 X 10-8M 3H-R5020.

Seven days after E2 implantation, PIT and HPS cytosol estradiol receptor levels were significantly reduced, while progesterone receptor levels were significantly increased, as compared to controls. Five days after E2 implant removal, PIT and HPS E2 receptor levels, and HPS progesterone receptor levels were indistinguishable from those C removed. Progesterone receptor levels also fell after E2 implant removal, but remained significantly higher than in C pretreated animals.

In conclusion, the 'long term' potentiation of lordosis by estradiol does not appear to be the result of a change in either estrogen or progesterone receptor levels in the HPS of the female rat brain.
1122 EFFECT OF KNIFE CUTS BETWEEN THE SUBFORNICAL ORGAN (SFO) AND ORGANUM VASCULOSUM LAMINAE TERMINALIS (OVLT) ON THE CENTRAL ACTIONS OF ANGIOTENSIN II (All). M. Ian Phillips, J. Phipps and Steven Bealer, Department of Physiology and Biophysics, University of Iowa, Iowa City, I A 52242.

The SFO and the OVLT have both been suggested as receptor areas for the effects of All. These effects include drinking behavior and blood pressure increases. Independent claims have been made that lesioning the SFO or the OVLT increases or decreases the responsiveness of a rat to All injected intraventricularly (IVT) or intravenously (iv). Since connections have been demonstrated between the SFO and the OVLT it is possible that OVLT lesions have produced the output pathway of the SFO. If the SFO is the only All receptor area, these tracts should abolish responsiveness to All iv or IVT, but if the OVLT area is also a receptor, cutting the connections would not impair the action of All.

To cut the SFO afferents a Halasz knife guide was lowered at 1° from the sagittal plane and turned 10° 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 cannulae. In seven rats with a cut, drinking to 100% All IVT was 8.0 ± 3.0 ml and in intact controls drinking was 7.2 ± 1.2 ml. There was no significant difference between the blood pressure response to All IVT in controls and experiments. All iv given by infusion over 30 minutes also produced drinking in experimental subjects. These results indicate that descending fibers from the SFO are not necessary for the response to central or peripheral All. There may be direct efferents from the OVLT. The results of pre- and postlesions of the SFO have shown that the effects of lesions in the OVLT area ("43IV") are not the result of interrupting efferent tracts 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 All. (Supported by grants from NIMH and NSF.)

1124 AFFERENT AND EFFERENT CONNECTIONS OF PUTATIVE PEPTIDERIC NEURONS OF THE PARAVENTRICULAR NUCLEUS (PVN). G.J.Pittman, N-W.Blume and G.E.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 thus undertaken an electrophysiological study of PVN cells in order to further define their afferent and efferent connections.

Experiments were conducted on pentobarbital anaesthetised Sprague-Dawley rats implanted with bipolar stimulating electrodes in the amygdala and midbrain central grey. Bipolar electrodes were also placed on the surface of the ME and/or across the PP stalk. Microprobes inserted by a ventral approach were used to record extracellular unit activity. A FDP 11/40 computer was utilized for analysis of spike discharge patterns. Antidiromic invasion techniques were used to identify efferent pathways from PVN. Antidiromic 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 antidiromic invasion from the PP to 93 PVN cells. A further 68 PVN cells, including 5 having phasic activity, displayed antidiromic invasion from the ME, thus supporting the existence of pathways from the ME to the PP and/or the PVN. This part of the population of cells appeared to be separate from that which projected to the PP. However, 13 of 136 PVN cells tested with both PP and ME stimulation displayed antidromic invasion from the ME sites. This would indicate the presence of simultaneous axon projections to both areas.

Different influences from the amygdala were evident in neurons with projections to the PP and in those projecting to the ME. S2 (n=90) of PP projecting neurons and 19% (n=48) of ME projecting neurons responded to ME stimulation after amygdala stimulation; the predominant initial effect was a decrease in excitability. In contrast, none of the PVN cells with projections to the ME showed orthodromic responses to midbrain stimulation. Thus the amygdala, but not the midbrain central grey 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)

1123 SERUM LH AND PROLACTIN CONCENTRATIONS IN INTACT AND CASTRATED RATS TREATED WITH S-HYDROXYTRYPTAMINE

Nancy S. Pilotte and John C. Porter. Dept. of OB-GYN and Physiology, Southern Methodist University, Dallas, TX.

The effects of S-hydroxytryptamine (SHT) on serum prolactin and LH levels in rats of both sexes have been investigated. The group of animals included orchiectomized as well as intact animals. The group of female rats included acutely and chronically ovariectomized animals as well as ovary-intact normal rats. Neuropeptide implants have been used to test the hypothesis that LH release 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 amino acids excite central neurones when applied iontophoretically or destroy neurones of the arcuate nucleus of the hypothalamus (AIH) when administered systemically. Since AIH 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 AIH neurones by neonatal treatment with high doses of Glu (ablatve approach) results in markedly reduced AIH concentrations of LH. In this study we investigated the possibility that there may be at least two subpopulations of AIH neurones - dopaminergic and cholinergic. In provocative experiments, subtoxic doses of Glu, i.e., doses that do not destroy neurones, 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 acid, GABA and taurine, or the DA receptor blocking agents to block NMA-stimulated release of LH. It is known from microelectrophoretic experiments that GABA and cholinergic. In provocative experiments, subtoxic doses of Glu, i.e., doses that do not destroy neurones, 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 acid, GABA and taurine, or the DA receptor blocking agents to block NMA-stimulated release of LH. It is known from microelectrophoretic experiments that GABA and cholinergic. In provocative experiments, subtoxic doses of Glu, i.e., doses that do not destroy neurones, 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.

Table 1. Mean Serum LH and Prolactin Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>LH (ng/ml)</th>
<th>Prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intact</td>
<td>43 ± 5.9</td>
<td>NS 39 ± 6.4</td>
</tr>
<tr>
<td>castrated</td>
<td>39 ± 5.8</td>
<td>&lt; .000 278 ± 54.0</td>
</tr>
<tr>
<td>Acutely</td>
<td>395 ± 38.9</td>
<td>.01 41 ± 8.9</td>
</tr>
<tr>
<td>castrated</td>
<td>230 ± 21.4</td>
<td>.01 360 ± 28.3</td>
</tr>
<tr>
<td>Chronically</td>
<td>492 ± 36.0</td>
<td>&lt; .05 274 ± 28.3</td>
</tr>
<tr>
<td>castrated</td>
<td>399 ± 21.2</td>
<td>&lt; .05 123 ± 37.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acutely</td>
<td>&lt;30</td>
<td>&lt; .01 76 ± 14.9</td>
</tr>
<tr>
<td>castrated +</td>
<td>E</td>
<td>65 ± 9.7</td>
</tr>
<tr>
<td>8</td>
<td>332 ± 84.3</td>
<td></td>
</tr>
<tr>
<td>Acutely</td>
<td>62 ± 10.9</td>
<td>NS 36 ± 9.2</td>
</tr>
<tr>
<td>castrated</td>
<td>79 ± 20.0</td>
<td>NS 77 ± 13.9</td>
</tr>
<tr>
<td>Chronically</td>
<td>364 ± 30.9</td>
<td>&lt; .01 18 ± 1.8</td>
</tr>
<tr>
<td>castrated</td>
<td>307 ± 46.0</td>
<td>NS 53 ± 11.7</td>
</tr>
</tbody>
</table>

* Mean and SE; N = 10

The results suggest that serum prolactin concentration can be increased by the systemic administration of SHT.
1126


The antidiuretic and cardiovascular effects of nicotine have been attributed to its action on central neural sites such as the hypothalamus, the limbic system (Milton & Tertson, J. Physiol. 241: 607, 1974) and the brainstem (Porsius & Timmermans, Brain Res. 47: 327, 1972) as well as peripheral neural sites in the cervical parasympathetic baroreceptors (Nadapapohornchale et al., An. J. Physiol. 227: 1216, 1974). Our studies of the unanesthetized monkey support the hypothesis of a neural linkage between vasopressin (VP) release and nicotine-induced behavioral events (Hayward & Pavusathupisik, Neuroendocrin. 21: 120, 1975). The present study is an attempt to clarify 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 carotid cannula for the first time in the absence of a femoral venous catheter for plasma VP. 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 (MAPB), heart rate (HR), pupillary size, salivation and cortical EEG and obtained blood samples from a femoral venous catheter for plasma VP. During a 10-minute i.v. infusion of nicotine (25-50 ug/kg/min), plasma VP rises to 5-15 fold above basal values in association with an immediate and transient MAPB rise at 50-170s. During 10 minute HR response to the deprivation of VP, there was an increase in salivation and an unchanged EEG. Bilateral cervical vagotomy did not change these responses to nicotine.

Hyponatremia and saline administration of the nicotine to the acute chronic cat 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 whereas behavioral responses and the cardiovascular response is not essential.

(Supported, in part, by Grants No. HS-13411 and JS-05696 from USPHS and by No. 197-78-A-3 from the North Carolina Heart Association)

1127


Estrogen is a highly potent sex hormone that is responsible for the induction of sexual receptivity. It is also well documented that estradiol pilgrims of the female rat, although both phenomena are estrogen dependent. The SEP and the MPOA exert a similar inhibitory effect on estrous behavior in both species. The SEP lesion was aimed at identifying the patterns of single-unit activity which was correlated with the inhibitory effect of this stimulus on the estrous behavior of the female. It was found that estrogen-dependent phenomena are regulated by the same animal and that these cells are likely to participate in the mediation of effective states.

Supported by NIMH Grant MH81506. General support provided by the Georgia Department of Human Resources.

1128


Sexual behavior in the cat entails a sequence of events in which the male initiates contact by grappling 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 is typically accompanied by vocalizations. The afterreaction does not elicit the afterreaction in anestrous female cats. The present work was aimed at identifying the patterns of single-unit activity which accompany the display of the cat's estrus-dependent behavioral responses. The first event in the afterreaction was associated with a decline in unit activity which was correlated with the inhibitory effect of this stimulus on the cat's locomotor behavior. Units to genital stimulation which 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. Generation of these units was observed before any change in behavior or neck electromyographic activity was apparent. Activity in anterior brainstem units was related to the overall pattern of genital clitoral stimulation. When genital stimulation did not elicit an afterreaction, as in anestrous cats or estrous cats which were sedated with ketamine HCl, there was no significant elevation in neuronal activity, the basal level of neuronal activity being unchanged. The present study was conducted to determine whether stimulation of the reproductive system was essential for the induction of these discharge patterns in this species.

Supported by N.I.H. Grants NS-12260 and NS-13746.

1129


The distribution of estradiol-concentrating cells in the cat brain was determined by autoradiography in two ovariectomized adult female cats 1 hr after i.v. injection of 3 mg of 3H-estradiol (NEB, 92 Ci/mmol). Heavily labelled cells were found in the bed nucleus (n.) of the stria terminalis, lateral septal n., peri-ventricular n., periventricular n., ventromedial n., tuberoinfundibular area, periventricular n., ventromedial n., tuberoinfundibular area, the cortical and basomedial nuclear of the amygdala, amygdalo-hippocampal area, periallgyaloid cortex, and ventral dentate gyrus. Labelled cells were also found in the islands of Calleja, n. of the diagonal band, medial septal n., the lateral, paraventricular, dorsomedial, and posterior hypo-thalamic nuclear, 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. 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 male and female cats. At 48-78 hours after the injection of 0.05-0.15 ul of 3H RFP in saline, neurons containing the granular reaction product were considered to be neuronal processes that are likely to be involved in the mediation of the possible neuroendocrine effects of estradiol in this area. HRP labelled neurons were found in most cases in anterior thalamic nucleus, periaqueductal gray, septum, and amygdala. Ventromedial, dorsomedial, tubero-infundibular, periamygdaloid 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 (<5um) to blood vessels and had processes directed toward the blood vessel stimulation. 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 estradiol. More research is needed to determine the role of these structures in the mediation of effective states.

Supported by NIMH Grant MH81506. 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 response to the onset of estrus but little LH in the morning (Caligaris et al., Endocrinology 89: 331, 1971). The following hypotheses could explain the absence of the morning rise in plasma LH: A) central monoaminergic pathways mediating the P-effect, B) reduced pituitary sensitivity to LH-RH, C) diurnal variation in the formation of obligatory 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 or 1200 h colony time and challenged with P or P-metabolites (5α-dihydro-P, 20α-dihydro-P, 3α-hydroxy-5α-pregnen-20-one) or with D-Ala2-Gly-His-Lys6-LHRH-ethylamide exactly 72 h later. The monoamine precursors DL-threo-dihydroxyphenylserine or 5-,6-dihydroxy-L-tryptophan (5,6-DHT) 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). Li and FSH release induced by the LHRH analogue and monitored over 4 h was significantly greater in the afternoon (LH: p=0.026; FSH: p=0.009). All P-metabolites tested increased LH release in the morning response: a) inactivity of central monoaminergic 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 MENESEPHALIC ELECTRICAL STIMULATION. Yasuo Sakuma* and D.W. Pfaff.

Estradiol-concentrating neureones are found in several cell groups thought to be involved in the regulation of female copulatory behavior and in a recent 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-wire 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, estradiol-primed female rats. Females were divided into 5 groups on the basis of lordosis 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<0.001) and in response to male mounting (P<0.01). Percent increases ranged between 53 and 150% of the pre-Experimental increase following 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, CG stimulation failed to facilitate lordosis. Both VMN and CG facilitation occurred on both sides of the brain.

1132 EFFECTS OF AGENTS WHICH DEPLETE SEROTONIN ON ULTRASTRUCTURE OF THE MEDIAN EMINENCE AND PITUITARY PARS INTERMEDIARIA. Linda C. Stahl and William B. Olds.

Adult ovariectomized rats were bilaterally implanted with silver wires to the median eminence and posterior hypothalamus. They were subsequently implanted with intraventricular electrodes. Lesions were made in the median eminence at various levels. They were subsequently treated with either VMN or POA stimulation. They were then returned to the appropriate ventricular site, and intraventricular administration of 5-HTP facilitated lordosis in response to manual stimulation (P<.01) and male mounting (P<.01). What distinguished the facilitatory response to CG stimulation from the effect of VMN stimulation was its fast time course. 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 50 µA, and is optimally induced by stimuli delivered at 50-150 Hz.

These results suggest that different cell groups with estradiol-concentrating neurons 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 lordosis 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.

1133 RADIOIMMUNOLOGIC LOCALIZATION OF Vasoactive Intestinal Polypeptide (VIP) IN INTRA- AND EXTRA-HYPOTHALAMIC SITES OF THE RAT BRAIN. U. E. Richie, M., E. S. Stanson, D. G. Gerhart, Dept. of Physiology and Pharmacology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The present paper describes the distribution of VIP in Wistar rats. The presence and quantity of VIP was determined by RIA in whole hypothalamus and the demonstration that VIP can act at the level of the hypothalamus to modulate anterior pituitary hormone release. We do not discuss the effects of VIP on the release of posterior pituitary hormones, since these problems have been recently reviewed. We present evidence that VIP facilitates lordosis in response to manual stimulation and male mounting. The VIP action was effective in adrenalectomized rats, in dexamethasone-primed animals, and in animals preloaded with exogenous progesterone. In contrast, POA stimulation suppressed lordosis behavior and facilitation occurred on both sides of the brain.

Rats were given implants bilaterally into the VMN and POA, and the VIP content of the hypothalamus was determined using a radioimmunoassay. The VIP content was highest in the VMN and POA. In contrast, VIP was absent from the lateral hypothalamus, the hypothalamus, and the pituitary. The VIP levels were significantly lower in the VMN and POA than in the POA and lateral hypothalamus. The VIP content of the hypothalamus was significantly lower in the VMN and POA than in the POA and lateral hypothalamus.

The VIP content of the hypothalamus was significantly lower in the VMN and POA than in the POA and lateral hypothalamus. The VIP content of the hypothalamus was significantly lower in the VMN and POA than in the POA and lateral hypothalamus.
1134 SUPRASELLAR CONTROL OF LUTEINIZING HORMONE (LH) SECRETION BY N-METHYL ASPARTIC ACID (NMA) J. Schenck, - and Theodore J. C. Schenck. * (SPON: James S. Nelson) Dep. 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 days) male rats at subtoxic doses (Price et al., N. S. Abs. 3357. 1977). Other analogues, such as glutamic acid and kainic acid, act similarly but their behavioral impotence and 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 groups of 10 4-day-avg) Sprague Dawley derived rats 7.5 min prior to sacrifice. Serum LH values (mean ± S.E.M., n=6), determined by radioimmuno­assay, for control rats were 446±116, 609±108 and 461±96 ng/ml, respectively. A suprassellar lesion is the presumed site of action of subtoxic doses of NMA since: 1) the LH surge is known to involve 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 100 μl were tabulated:

<table>
<thead>
<tr>
<th>Compound in Medium</th>
<th>LH in Medium/100 μl flash/3 hr S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>LHRH 10-5M</td>
<td>150 ± 10*</td>
</tr>
<tr>
<td>NMA 10-5M</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>LHRH 10-5M + NMA 10-5M</td>
<td>140 ± 8*</td>
</tr>
</tbody>
</table>

These results demonstrate that NMA does not induce LH secretion by direct action at the pituitary, but rather presumably acts at a suprassellar locus. Accordingly, NMA will undoubtedly provide a useful tool to the neuroendocrinologist for elucidating the neural control of the pituitary LH secretion since it: 1) acutely (within minutes) elevates serum LH in the young adult (54 days) as well as immature (25 days) male rats, but 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 parenterally at subtoxic doses. This research was supported in part by Project Grants from the Ontario Mental Health Foundation (O.M.H.F.) and an O.M.H.F. Scholar.

1135 THE DISTRIBUTION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN THE FETAL GUINEA PIG BRAIN. H. Schenck-Fukuda* and A.J. Silverman. Dept. Anat., Columbia Univ., New York, NY 10023. Brains from littermate male and female fetal guinea pigs were studied using a modified antigens of LH (265 μg/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 of the ME. There was observable at 8 hr post-injection, 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. Therefore, the monoamine oxidase inhibitor, pheniprazine (10 mg/kg i.p. 14 hr prior to sacrifice) did not reverse the acute effect of reserpine on NP and VP. The paraventricular nuclei (PVN) was determined as the source of NP and VP in the external layer. Bilateral lesion of the PWN eliminated all of the external layer of NP and VP. Bilateral lesion of the supraoptic nucleus of the hypothalamus 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 those of the internal layer by their pattern of distribution. These differences may well be related to the sites of origin of the fibers in the lateral hypothalamus. There are likely to be two types of arcuate neurons which give rise to these different distributions. The first type is located in the external layer of the ME and is responsible for the distribution of NP and VP fibers seen in the external layer of the ME. The second type is located in the internal layer of the ME and is responsible for the distribution of NP and VP fibers seen in the internal layer of the ME. The differences in the distribution of NP and VP fibers in the external layer of the ME may be due to a direct effect of the drug on neuroendocrine neurons and not reflect reserpine's action on the storage of monoamines.
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 hypotension. These sites are the subfornical organ (SFO; Mangia­

1138

pame and Simpson, Neurosci. 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 the true proximal of the third ventricle (III V). SFO cannulae did not perforate ventricular ependyma. A second group each received a cannula terminating within the OR. Periventricular loci or III V, ependyma was ruptured only at the classified site of injection. Measures of water intake and mean arterial 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 amnesthetized animals. Maximal efficacy for both A II effects was observed when the can­

nula terminated in the SFO rather than in tissue proximal to 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. The site of action of A II in III V. Further, OR injection of these doses of A II provoked a pressor response which frequently commenced only after drinking behavior. The pressor effect of OR injection of A II was second­

ary 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 those doses of A II 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 angiotensin II action in rats.

Supported by HL 21799 and HL 21500.


Previously we have reported (Neuroscience Abstracts 3: 1161, 1977) that the medial preoptic nucleus (MPN), a small periventric­

ular nucleus caudal to the organum vasculosum of the lamina ter­

minalis, is necessary for estrous cyclicity in rats. In the present experiment, function of the MPN, as well as adjacent struc­

tures, for the release of gonadotropins during the critical period of proestrus was studied. The first day of proestrus was examined. In regular 4 or 5 day cy­

clic rats brain lesions and stimulations were performed on pro­

estrus day, and the number of ovulation of the ampulla on estrous morning. If the ampulla was dilated, it was dissected out and the number of ova counted under a micro­

scope. If the ampulla was not dilated, the ovary was not removed and vaginal cyclicality was followed postoperatively.

Experiment I: Bilateral electrolytic lesions with a platinum electrode were made in the MPN, the perivascular part of the medial preoptic area (PMPD, just dorsal to the organum vas­

culosum of the lamina terminalis, usually causing a 10% or less of the lesions of the medial preoptic area (900-1000). The lesions consisted of the lesioned (0 of 11, 0 of 11 rats ovulated, respectively), while lesions of the SCN failed to block (4 of 4 rats ovulated). How­

ever, in the animals with PMPD lesions, subsequent cyclic ovula­

tion was indicated by resumption of vaginal cycles, while most of the animals with MPN lesions showed persistent vaginal estrus. After lesioning, all animals were placed in cages with MPN- or PMPD-lesioned, electrochemical stimulation (ECS) was applied to the medial preoptic area (MPN) under ether anesthesia on proestrus morning (1400-1500). Amplitude of 15-75 µA to the MPN through stainless steel electrodes for 60 sec consistently induced ovulation in the pentobarbital blocked proestrus rats (all 8 ovulated). ECS to the MPN with the same current resulted in ovulation in 6 of 7 MPN-lesioned animals (1000 µA for 30 sec), ECS of the MPN-induced ovulation in 3 of 11 MPN-lesioned animals and 6 of 7 PMPD-lesioned animals. Sham ECS in the MPN- or PMPD-lesioned animals was ineffective in inducing ovulation in the pentobarbital blocked proestrus rats (all 8 ovulated).

It is concluded that 1) the major portion of the neurons neces­

sary for the release of LH-RH on the afternoon of proestrus origi­

nate in the vicinity of the MPN, but not the SCN; 2) most, but not all, of the neurons in the MPN 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 RO1 HD1355-01.)

ROLE OF ACETYLCOLINE AND ANGIOTENSIN IN THE OSMOTIC CONTROL OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOPHYSIOGRAPH SYSTEM. Celia D. Sladek and Robert J. Joynt. 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 corticoste­

rone to synaptic plasma membrane (SPM) was examined. Male rats were adenectomized for 3 days and perfused with saline prior to brain dissection. The cell cytosol (105,000 g supernate) and SPM were prepared from the hippocampus, hypothalamus and cortical cortex by a centrifugation procedure using discontinuous sucrose density gradients. As controls, rat direct cortical terminals were taken at 4 C, SPM was applied to DEAE-cellulose filters and washed free of unbound 3M-steroid. The binding of 3M-cortisosterone to cell cytosol (10-9 M) was effective in blocking VP release by the organ cultured hypothalamo-neurohypophyseal system (HNS; Sladek and Joynt, Neurology and Anatomy, Univ. Rochester School of Medicine, Roch­

ester, N.Y. 14642

ester, N.Y. 14642

estorin (VP) release. Each of these organotypic explants includes hypothalamic tissue distal to the organum vasculosum of the lamina terminalis. Sarcin, an All antagonist, blocks osmotically stimulated VP release by the HNS (Sladek and Joynt, Neurosci. Absts. 3:357, 1977). These data suggest that All also modulates osmotic control of VP release. The relationship between All and cholin­

ergic modulation of VP release was examined by assessing the effect of hexamethionin on All stimulation of VP release, and the effect of saralasin on cholinergic stimulation of VP release. Hexamethionin (10-6, 10-5, 10-4 M) was ineffective in blocking VP release by All (10-5 M), Saralasin (10-6 M) did not block stimula­

tion of VP release by either ACH (10-6 M) or nicotine (10-5 M).

These findings suggest independent All and cholinergic mechanisms controlling VP release. The role of cholinergic and hexamethionin in blocking osmotically stimulated VP release indicates some interaction between these regulators of VP release. A HR that consists of a VP producing cell possessing nicotinic-cholinergic receptors and a separate osmoreceptive cell which communicates with the VP cell (by means of an interneuron not by means of the VP cell) is proposed. From the information in the osmorecep­

tor is modulated by All possibly by modulating release of or acting as a neurotransmitter from an interneuron. (Supported by NIH grant AM 76197 and Research Career Award is-00259.)

BINDING OF CORTICOSTERONE TO SYNAPTIC PLASMA MEMBRANE FROM RAT BRAIN. A.C. Towle* and F.Y. Sze (SPON: B.E. Ginsburg). Dept. of Biobehavioral Sci., Univ. of Connecticut, Storrs, Ct. 06268

Specific binding of glucocorticoids to brain SPM suggests that the steroid hormone may act on nerve terminals by possibly their release. Compared with 3H-corticosterone binding to cell cytosol (10-5 M) was ineffective in blocking VP release by All (10-5 M) and to soluble content of symaptosomes (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 3M-cortisosterone with 1,000-fold unlabeled steroid.

From ligand bound/mg protein vs. log [ligand concentration] plots, specific binding of 3M-cortisosterone to SPM obtained all 3 brain regions shows a similar sigmoidal curve, reaching saturation of dissociable binding sites at 3 x 10-7 M. Compared with 3M-cortisosterone binding to cell cytosol (saturating at 2-6x10-5 M), the affinity of the steroid binding sites in SPM approach the affinity of 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 than the cell cytosol binding(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 symap­

tosomes soluable content shows only minimally detectable binding capacity, indicating that in brain terminal the cytosol binding protein(s) may be confined to the perikarya.

Specific binding of glucocorticoids to cell plasma membrane has been described in the vicinity of the MPN, but not the SCN; 2) most, but not all, of the neurons in the MPN 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 RO1 HD1355-01.)
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. In general, only in endogenous binding only in the pituitary. Four regions of the brain were examined: pituitary, hypothalamus, cerebral cortex, and hippocampus. In the unstressed rat the highest levels of cytosol binding were observed in the cerebral cortex, followed by the pituitary and hypothalamus. In general, only 50% of cytosol binding sites were occupied in the basal animals, indicating a high degree of binding. The observation of area differences would suggest specific anatomical sites as having significant roles in the stress response.

In the unstressed rat the highest levels of cytosol binding were observed in the cerebral cortex, followed by the pituitary and hypothalamus. Total sites were also higher in the POS than in other brain regions with the exception of the pituitary. Stress produced an increase in 5-HT binding only in the preoptic area (POA), including the area medians (AM); however, a significant increase in binding was observed in the POA. All brain regions except the pituitary showed a decrease in 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 07471 to BBT.


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., 1969) have suggested that the neuroendocrine cells of the PVN are relatively resistant to stimulation by Golgi methods, by systematically varying block size, animal age, chemical concentration, and times in solutions in 207 rats, a consistent method has been found which yields the highest percentage of magnocellular PVN neurons of 29 rats. To verify the identity of magnocellular PVN neurons, soma size, 1 µ plastic sections containing the nuclei, and 4C, were used to investigate. Cells containing silver chrome were compared with horseradish peroxidase filled neurons and with ultrathin sections to confirm these results. Two or three primary dendrites issue from PVN soma. 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 irregular outer membranes, with dilations interspersed with narrow constrictions; since these were also visible with EM, it is unlikely these are Golgi artifacts. Although exceptions were noted, dendrites tend to stay within PVN borders, whether these are functional or not. Dendrites were also seen with very irregular membranes, indicating a high degree of binding. These results suggest that, although the MR projects both to the mediod 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.

SEX DIFFERENCES IN THE RESPONSE OF HIPPOCAMPAL CA1 PYRAMIDS TO GONADAL STEROIDS: EFFECTS OF TESTOSTERONE AND ESTRADIOL ON THE IN VITRO SLICE PREPARATION. Richard H. Vardaris and Timothy J. Taylor, Kent State University, Kent, Ohio 44242 and Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44246.

Monosynaptic extracellular EPSPs and population spikes were recorded from hippocampal CA1 pyramidal cells following stimulation of the Schaffer collaterals in explanted rat hippocampus. The major criteria used to establish appropriate physiological status of the preparations were: (a) presence of paired-pulse facilitation; (b) waveform similar to those observed in intact preparations; (c) presence of paired-pulse facilitation with EM.

Testosterone (T) and 17-beta-estradiol (E) 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—T—E or T—E—T. Slices were obtained from estrousand 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, the pattern was reversed, with E depressing responses and T enhancing responses; whereas in females the pattern was reversed, with E, depressing responses and T enhancing responses. This was accomplished by adding the steroids to the recording medium, the slices being electrically stimulated with paired-pulse (E) and single (T) stimuli, the two having different receptors, as has been reported for other tissues.
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 normal 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 cortical amygdala (AMYG). Vaginal cycles were unaffected. 1 to 2 mos. post-lesion rats were ovariecotomized. Tests for LH response began 3 weeks later. Each rat received a series of 3 doses of EB, 7 ug/kg before P. While all doses of oil-treated control values at 77 hrs, regardless of lesion group.

AMYG, typically normal in behavioral responsiveness, do not show facilitated LH response to 70 ug/kg before P. While all doses of oil-treated control values at 77 hrs, regardless of lesion group.

Tests. Comparisons presented were significant at p < 0.05 or less.

Thus, BULBX do not deviate from the SHAM pattern of LH values for AMYG were 95% of Pre-EB, at 5,29,53, & 77 hrs after each dose of EB. Half of each group was always injected with 2mg P 2hrs 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, regardless of lesion. At 29 hrs, higher doses of EB were more effective than 7ug/kg in enhancing LH.(OVX) levels ranging from 10-20ng/ml. While all doses of oil-treated control values at 77 hrs, regardless of lesion group.

Thus, BULBX do not deviate from the SHAM pattern of LH values for AMYG were 95% of Pre-EB, at 5,29,53, & 77 hrs after each dose of EB. Half of each group was always injected with 2mg P 2hrs 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, regardless 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 655ng/ml; BULBX, 144% of Pre-EB, at 800ng/ml. Values for AMYG were 95% of Pre-EB, at 515 ng/ml. Values for AMYG were 95% of Pre-EB, at 515 ng/ml.

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, Deps. 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, ovariecotomized (OVX) conscious rats bearing chronically implanted 3rd ventricular cannula were injected with 2 ml saline containing varying doses of VIP and plasma LH, FSH, Gn, GH, TSH and FSH levels were measured by RIA in jugular blood samples drawn through an indwelling atlantic 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 FSH levels were elevated by 40 and 100 ng doses; however, the highest dose of 500 ng had no effect on plasma LH or FSH levels. Plasma Gn 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 FSH 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% O2 and 5% CO2, with doses of VIP ranging from 10 ng to 1000 ng produced a dose-dependent increase of TSH and PRL as well as TSH and PRL as well as other pituitary hormones. 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 RAM0073, HD09988 and from the Ford Foundation to S.M. McCann and H.J. Smith and to S.A. Wurtman.
NEUROETHOLOGY
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 wings; 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 orthopteran reflexes which serve to assure smooth, coordinated, oriented flight.

A similar set of reflexes exists in certain flightless grasshoppers. Barysettis sp., a brachypterous, subtropical grasshopper, possesses no hindwings, and is inhibited by tarsal contact with any firm substrate. Tethered individuals respond to initiation of a windstream over their bodies by throwing their legs up into a stereotypic flight-like posture, and under simulatedyaw conditions, perform asymmetrical orientation movements of their hind limbs. Barysettis 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 Barysettis, 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, 1964, Nature, 204:287-288).

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 hair. It is perhaps significant that 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. This model system when these fish were compared with normal control fish


Bilateral radiofrequency lesions (x current=0.5mA; x duration 75 sec using glass insulated platinum-iridium microelectrodes with exposed tips of 100 or 250mV courtesy of F. Haer Co.) of the anterior hypothalamus-preoptic area (AH-POA) abolish male courtship and agonistic behaviors in both intact and castrated/andro­

toid treated Anolis carolinensis. Lesions caudal and dorsal to the AH-POA also cause a significant decline in the behavior of intact animals. Histological examination of testes showed that lesions including and rostral to the AH-POA cause testicular col­

collapse and regression to initial stages of spermatogenesis, in­
dicating interruption of gonadotropin secretion. Castration and subcutaneous testosterone implants restores courtship and agon­

istic behaviors in rostral and caudal lesion groups to prelesion levels, while AR-POA lesioned animals remain at their low be­
havioral levels. Neither AR-POA or control lesions cause signif­i­

icant changes in animals' body weight.

Free testosterone (T) or testosterone propionate (TP) implants (ejected pellet technique using 33g hypodermic tubing) placed unilaterally in the AR-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 AR-POA. The mean response latency differed for the two hormones used: T averaged 3 day; while TP implants 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 NIH Research Scientist Develop­ment Award 1 KO2 MB00135.


Weakly electric fish produce an electric field in their near vicinity. objects within this field cause potentials of a magnitude 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, gymno­
toid electric fish with pulsed EODs shorten their EOD intervals in response to pulses that scan their field. We also examined the negative effects of long series of pulse coincidences, which are most detrimental to electrolocation. The JAR in pulse species strongly resembles that in wave species (Bullock et al, Physiol. Neurobiol., 1:247-256).

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 unpaired preparations demonstrate that the JAR is controlled by electroreceptive input alone, without reference to an internal electric organ pacemaker-related oscillator. A suf­

ficient stimulus input consists of a train of strong, EOD-like stimulus pulses ($t_1$), which mimic the animal's experience of its own EOD, and a train of small pulses ($t_2$) of slightly different repetition rate, which mimic EODs of a neighbor. Correct behav­

ioral responses require $t_2$ repetition rates comparable to normal EOD sequences; $t_1$ repetition rates are not sufficient. The JAR must closely resemble those of the animal's EOD. These features are of little significance for $t_1$ pulses which, while scanning $t_2$ pulses, only provide a small perturbation of electroreceptive feedback from $t_1$ pulses.

$t_1$ pulses must also be of sufficient intensity to recruit electroreceptors, of which there are two types. Pulse markers fire for one spike at a constant latency after the $t_1$ latency and do not respond to scanning by $t_2$. Burst duration coders fire a burst of spikes following each $t_1$; the burst pattern changes during $t_2$ 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.

THE MAUTHNER SYSTEM MEDIATES DIRECTIONAL RESPONSES TO LOCAL VIB­


Electrophysiological experiments show that the Mauthner (M-) cells of zebrafish larvae initiate startle responses to local vib­

rational stimulation produced by low-frequency, axial excursions of the roccapillary organ, stimulated by the body (Eaton et al., 1977, J. Neurobiol., 8:151). Larvae transformed by X-rays result in stimu­

lation results in startle behavior involving a contraction of the contralateral body musculature, such that the animal gives a direct response. Avoidance responses require the M-system to respond to scanning by S1. Correct behavioral responses are 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 stimu­

lation. 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 are the two ways that animals could accomplish this? One way is through 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 bilateral stimulation, thus illustrating the importance of audition for some types of tattle behavior.

Supported by NSF Grant BNS 77-08685 to CBK and an Intramural Grant to RCE from Univ. of Colorado.
From quantitative dummy experiments with rectangu-
lar moving stimuli we know that toads Bufo bufo prefer
"worm-like" prey objects (stripe axis oriented parallel to
the movement direction) and avoid "antworm-like"
objects (stripe axis oriented perpendicular to the move-
m ent direction). The ability of toads to distinguish
worms from antworms 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 li-
mits). 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
objects fine-field s, pattern-oriented receptive fields in
the stimulus area but mainly to its expansion
perpendicular to the direction of stimulus movement.
Tectum neurons can be characterized by response char-
acteristics to configurational parameters, which
reflects a good approximation the probability to
match the stimuli with the stimuli in the tectum of
non-mammals. Tectum neurons receive excitatory inputs
to tectum 1 neurons, and there is evidence that they receive in-
hibitory inputs from TP-neurons. It is thought that
tectum 2 neurons are involved in the system which triggers
the prey-catching orienting movement, once a par-
icular level of neuronal activity has been reached.

This is supported by the occurrence of an increase
stimulation of the optic tectum in freely moving ani-
mals.

**THE JAMMING AVOIDANCE RESPONSE (JAR) IN EIGEMANNIA:**

**MOTION DETECTION IN A TWO-DIMENSIONAL STATE PLANE.**

Walter Heiligenberg, Curtis L. Baker and Joanne Matsubara.

Scipps Institution of Oceanography, UCSD, La Jolla, Cal. 92037.

The JAR in the electric fish Eigenmannia is an attempt to move
the electric organ pacemaker frequency away from similar frequen-
cies of a jamming stimulus, normally the electric organ discharge
(EOD) of a conspecific. The JAR requires simultaneous electro-
reception of the EOD's own EOD and a jamming stimulus
(Bullock et al., J.comp.Physiol. 11, 1-48). Correct JARs
can be elicited if the EOD, silenced by curarization, is replaced by a
sinewave stimulus, S, which sufficiently mimics the natural EOD
frequency tens of Hz different from that of the pacemaker. The JAR
therefore appears to be driven by electroreceptive afferen
ces alone, i.e., without an internal reference to the pacemaker cycle.

The addition of the EOD or its substitute, S, and a jamming
sinewave stimulus, S, results in a sinusoidal signal, s, whose
amplitude and phase with regard to that of s are modulated at the base
frequency, f, the difference between the frequencies of S and s.

Whereas beats modulation is identical for positive and negative
frequencies, phase modulations are opposite of one another. Amplitude and phase are coded by different types of
electroreceptors, P- and T-units, respectively (Scheich et al.,
J. Neurophysiol. 35, 39-60), and simultaneous evaluation of their
two types of activity should yield the magnitude and the sign of the
A. By plotting amplitude and phase as parameters in a
two-dimensional state plane, closed graphs are obtained which are
reproduced times per second. The direction of motion of a point
in this graph is determined by the sign of the A. Evidence is
given that the animal detects motion in this plane by a
mechanism comparable to motion detection in the realm of vision.

**DOES THE MAUTHNER NEURON MEDIATE UNIQUE BEHAVIOR?**

Charles S. Kimmel, Susan L. Powell, Dept. Biology, Univ.
Oregon, Eugene, OR 97403, and Robert C. Eaton, Dept. E.P.O.
Biological, Univ. Colorado, Boulder, CO 80309.

On the basis of both electrophysiological and behavioral evidence
we proposed that the Mauthner (M-) cells of larval zebrafish initiate fast start behavior in which the first
movement is a short-latency (410 ms) stereotyped con-
traction that causes the body to form an "S" shape
(Eaton et al., J. Neurobiol. 8, 151, 1977). This initial
phase is believed to represent the direct output of an M-cell and its muscular innervation. We have evidence with deletions of the M-cells induced by irradiation at the
gastrula stage (Kimmel et al., Development. 86. 62: 526,
1978) to learn whether presence of the M-cell is uniquely
correlated with presence of this behavior.

High speed (400 fps) 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 a 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 (mean latency: 0.61 body
lengths in 10 ms) whereas those to the opposite side (18 of 37 vs. 4 of 30). All of
the short-latency responses to the M-cells response were C-type fast starts (mean displacement: 0.61 body lengths in 10 ms), whereas those to the opposite side were heterogeneous and significantly less in mean strength (0.32 body
lengths in 10 ms). Only 1 of these starts to the side
missing the M-cells was as strong as the weakest short-
latency response observed to the side on which the M-cell 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, whereas other neural circuits are sometimes capable of producing apparently identical responses. It is not known whether these other circuits arise as a result of the M-cell, and even though such compensation may have happened, it was insuf-
ficient to match the performance advantage which occurred when the
M-cell was present.

Scripps Institution of Oceanography, UCSD, La Jolla, CA 92037.

Weakly electric fish can be classified on the basis of their electric organ discharges (EODs) into pulse and wave species. While 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 minimize 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 white 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 it 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 conditions, Sternopygus (given stimuli of sufficient intensity), A. sternarchoides and A. eigenmanni are most vulnerable to stimulus freq. which differ slightly from their own.

3) When certain wave species are submitted to electric stimuli, unnaturally strong sinusoidal stimuli, as great as 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 it 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.

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 neocortex, for the expression of most forms of space-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. Behavioral 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 board building, tunnel blocking, use of a urination post, daily activity rhythm, play-fighting, territorial aggression, display of reproductive behavior in intact females, and courtship behavior in intact males. The experimental males had some motor difficulties during mating and required twice the normal time for intromissions and ejaculation, but successfully impregnated females. A female with bilateral absence of neocortex mated, became pregnant and reared her young. Experimentally deprived animals displayed more stereotypic than the normal controls.

Some animals had varying amounts of damage to the midline structures. In the case of the normal hamster, there was a corresponding reduction of the neothalamic nuclei. The results of this study reveal that animals with intact midline and limbic structures, but lacking a normal neocortex, are capable of giving expression to a wide range of species-typical behavior.

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 gold Syrian hamsters, selected for aggressiveness and implanted with a chronic, moveable, bipolar concentric electrode cemented into the left ventral tegmental area (Smith and Jones, 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).

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 stimuli 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 songs and to orient to them. Freedly walking females were presented simultaneously with two electronically synthesized song models which differed only in the arrangement of sound pulses. The females showed a clear preference for the heterospecific. The females were able to 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 disrupted, was rejected by T. oceanicus. These results argue against a hypothesis that females possess a copy of the central neuronal pattern generator which 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 interpreted as heterospecific 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 teetered flying females were presented with two sound pulses, arranged in a stereotyped sequence. This assay measures the relative attractiveness of the two songs. This flight assay has so far yielded results similar to those obtained with walking females. Furthermore, the females are more sensitive and more reliable, and has the advantage of providing a simple, well-defined output which reflects song preference. This laboratory should facilitate physiological analyses of song recognition mechanisms.

THE ROLE OF ENVIRONMENTAL CONCENTRATIONS OF MINERALS AND PITUITARY HORMONES IN REGULATING REPRODUCTION OF THE PARADISE FISH, Macropodus opercularis. Trudy Viliars, Joseph Morris* and David Seaver*. Saint Mary’s College, Notre Dame, IN 46556.

Environmental cues such as temperature and photoperiod play a major role in the reproductive cycle of paradise fish. Cues such as mineral content ('hardness') of the water following rainfall or drought might also be expected to affect reproductive readiness. In some freshwater species, the effect of water hardness is known to be mediated by changes in metals ion content. The present study was undertaken to determine the role of water 'hardness' on nestbuilding and spawning of the paradise fish and 2) the role of pituitary hormones in mediating such a response.

The paradise fish is a fairly specialized species which has an elaborate reproductive sequence including a courtship of 1 to 4 male nestbuilders and one female. The sequence includes the following steps: 1) the effects of water 'hardness' on nestbuilding and spawning of the paradise fish and 2) the role of pituitary hormones in mediating such a response.

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 introduction. 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.

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 introduction. 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 PERCENT SPAWNING
10 'hard' 10% 90%
11 medium 90% 10%
12 'soft' 100% 0%
13 'hard' 10% 90%
14 medium 90% 10%
15 'soft' 100% 0%

Preliminary histological analysis of the pituitary indicates a reduction of activity in the acidophilic cells of the pars distalis (Bray, 1970). In Acidophilic and basophilic cells such cells are inhibited by environmental salinity and play a role in osmoregulation (Schreibman et al. Am. Zool. 13: 719, 1973). In the present experiment pituitary hormone levels in advanced based and stimulated osmoregulation (Machemer, Z. Tierpsy. 28: 33, 1971). In the course of evolutionary 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
MODULATED RECEPTOR HYPOTHESIS FOR THE INCREASE IN DESSENSITIZATION OF THE ACETYLCHOLINE RECEPTOR BY ANESTHETIC TYPE AGENTS. Roger Amo lying to the desensitization site, the receptor channel only after the receptor has been activated by ACh. After binding closes at its normal rate but the receptor is converted to an histrionicotoxin; 1 x 10^{-6} M and no effect and 5 x 10^{-4} M benzocaine; no effect and 5 x 10^{-4} M heptanol; 1 x 10^{-7} M and 1 and to cause antagonism of extrajunctional acetylcholine (ACh)

This 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.

SUPPRESSION OF ORIGINAL NERVE INPUTS TO A MAMMALIAN SKELETAL MUSCLE BY A FOREIGN MOTOR NERVE. J. L. Blaby 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 extrajunctional 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 4-20 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 the original synaptic and the establishment of foreign ones is a graded phenomenon. We believe, on the basis of cholinesterase staining and rise times of end-plate 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 is not a result of injury 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.


Neuromuscular contacts were formed in vitro by adding dissociated neuromuscular 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 aggregates or clusters which have putative acetylcholine receptors (AChRs). In innervated cultures, these clusters were found in the muscle membrane along the nerve contact (Piluso, 1974, 1978). In the present developmental study, most nerve-muscle contacts in one-day co-culture were marked by 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 ACh 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. In the extrajunctional muscle membrane containing synaptic vesicles, mitochondria and microtubules. The post-junctional membrane sometimes formed caveolae. Beneath the membrane there were occasional accumulations of dense material in small patches. The junctional cleft was narrow, about 100 Å wide. In several instances there were very close junctions between the pre- and post-junctional membranes. 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. In another day co-culture, the endplates were observed in a study in the same system, using α-bungarotoxin conjugated to horseradish peroxidase, to detect the localization of AChRs in the post-junctional membrane (Iversen, 1981). (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 H.R. Cohen. Dept. of Physiology, McGill University, Montreal, Canada.

Previous studies have indicated that innervation of the myotomes in Xenopus laevis begins before stage 17, 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., J. Cell. Biol. 74:217, 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 radioautography and electron microscopy with radioactive iodine or with fluorescent dye (tetramethylrhodamine). Specific uptake of radioactive toxin was demonstrated as early as stage 32. The number of radioautographs obtained increased progressively with age throughout the period of development studied (up to 1 month). The distribution of these sites was examined in hydrocarbon preparations of Epon and cut longitudinally. At all stages of development, large grains were observed throughout the entire length of the myotomes. From the ends of the myotomes to the 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, 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 dense than their occurrence at later stages. 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 these 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 consistent with the notion of a local interaction between the axon and muscle cell that induces the accumulation of acetylcholine receptors at developing synaptic sites in vivo.

(Supported by NSC of Canada)

1170 PHARMACOLOGICAL EXPERIMENTS ON FROG MUSCLE USING AN ELECTROPHORETIC VOLTAGE CLAMP TECHNIQUE. J. del Castillo and F. Proctor. Lab. of Neurobiol. and Dept. of Physiol. Sci., U.C. Irvine, University of Puerto Rico, San Juan, P.R. 00936.

The conventional technique of electrophoretic drug application (Nathan et al., Fed. Proc. 41:1951, 1953) at 50 to 100 µA/mV (physiol. 128:157-161, 1955) has been employed successfully to map receptor distribution and to determine, both qualitatively and quantitatively, the sites of the receptor population. However, it has failed to generate significant information on the characteristics of drug-receptor reactions. Indeed, the electrophoretic technique is so inefficient 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. Thus, the depression of drug concentration introduced by those pulses are not known and therefore different reaction kinetics cannot be analyzed.

To use the electrophoretic technique to 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 feedback loop, by the membrane potential (del Castillo and Proctor, 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 time at 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 been employed to study the interaction between agonists and antagonists.

*Supported by USPHS grants Nos. NS-14938, NS-07464 and RR18082.

(Contribution No. 84, Laboratory of Neurobiology)


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 those agents are effective in altering the time sequence of progressive degenerative changes or the characteristics of morphological alterations evident in motor nerve terminal degeneration. As these events have been well documented in the rat phrenic-nervous diaphragm, this preparation was chosen for study.

Tramcinolone 80µg/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 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 the nerve containing degenerating nerve terminals 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. In 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 terminals become fragmented. Electron dense membrane fragments invade the site of the terminal. In triamcinolone-treated rats, 18-20 hrs after phrenicotomy approximately 35% of terminals sampled were normal degenerative form. Although evidence was present in other end-plates were primarily of the axonal terminal organization; the terminal was not fragmented. Fragmentation of the nerve terminals becomes evident 2 days after denervation in triamcinolone-treated rats. At this time in non-treated rats, all end-plate sites are replaced by the Schwann cell.


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 portions 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 neurons. 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 have been detected in peripheral nerve. Since cellular localization of 16S ACHE may be important in understanding nerve-muscle trophic interactions, we re-examined the enzymatic 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 (BM284C51 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), presumptive motor, cranial XII), vagus (sympathetic efferent), and sciatic nerves (peripheral, mixed motor-sensory), hypoglossal (cranial XII), and spinal dorsal 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 6.55 molecular form was found only in spinal cord and peripheral nerve. This may be a reflection of 6.55 ACHE specificity for nerve-muscle trophic interactions, to the degree of receptor subtype and state of aggregation or transformation of the other ACHE species.

Results indicate that 16S ACHE is not only present in muscle endplates, but it 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 the peripheral nerve. This might be related to neurotrophic regulation of neuromuscular junction ACHE, since neurotrophic factor might provide a source for the endplate enzyme. Current experiments are concerned with the stains and peripheral nerve. (Supported by the Muscle Dystrophy Assn. and the Medical Research Service of the Veterans Administration.

368
DAMMIT EFFECTIVENESS IN FROG CUTANEOUS PECTORIS AND SARTORIUS

DIFFERENT COMPONENTS OF BLACK WIDOW SPIDER VENOM MEDIATE TRANS-

FIBERs WHERE THE MORPHOLOGY AND PHYSIOLOGY OF IDENTIFIED

PROPERTY MIGHT CONSTITUTE AN INDEPENDENT MECHANISM FOR INCREASED

MUSCLE OF RANA PIPiens AND R. CATESBEIANA ARE SUBTHRESHOLD TO

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.)

JUNCTIONS. Albert A. Herrera* and Alan D. Grinnell.

INNERVATED FIBERS. Albert A. Herrera* and Alan D. Grinnell.

CRUDE BLACK WIDOW SPIDER VENOM (BWSV) CAUSES A MASSIVE INCREASE IN

EMPP-FLUORESCING RAISED OOF 1 Mm AND DIAMETERS OF BWSV IT

HAS PREVIOUSLY BEEN DETERMINED THAT (1) A 130,000 MW COMPONENT,

LATROTOXIN FORMERLY CALLED B-

THE LENGTH OF VESICLES IN THE TOTALLY DEPOLARIZED TISSUE, AS

DETERMINED BY LENS FLAME PROCESSING, IS REDUCED BY 30% IN THE

PLEXUS, WHICH IS ALSO REDUCED BY 30% IN THE

BRAIN. THE CALCULATION OF THE NUMBER OF QUANTAL CONTENTS (E)

IN VARIOUS NEUROMUSCULAR JUNCTIONS IS A COMMON METHOD TO

DETERMINATION OF THE EFFECTIVENESS OF NEUROMUSCULAR JUNCTIONS.

MITTER RELEASE AT VERTEBRATE AND CRUSTACEAN NEUROMUSCULAR


Crude black widow spider venom (BWSV) causes a massive increase in

mepp frequency at vertebrate neuromuscular junctions, a similar increase in mepp frequency at crustacean neuromuscular

junctions, and can release a number of transmitters from mouse cerebral cortex and raising purifications of BWSV. It has previously been determined that (1) a 130,000 MW component, α-latrotoxin (formerly called B-

latrotoxin) formerly called B-

α-latrotoxin (10 µg) however produces no effect.

In contrast, at the frog neuromuscular junction, where 1-4 µg of

α-latrotoxin in 3 ml bath causes a rapid increase in mepp

frequency, fraction E has no effect.

These results demonstrate that the effects of BWSV at verte-

brate synapses and lobster neuromuscular junctions are mediated

by different components. Consequently the mechanism of

BWSV action at vertebrate and at crustacean synapses may be

different.

Electrophysiological studies were conducted on the isolated sciatic nerve-sartorius muscle preparation of the frog Rana pipiens treated with the principal component of cannabis, 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. The lowering potentials of THC treated fibers did not differ from controls. Records taken at non-junctional regions showed that single supramaximal nerve stimuli failed to evoke a propagated action potential (AP) in 7 out of 16 fibers. In 60% of these non-neural 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 3 X 10^-6 M depresses the ionic conductance mechanism 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 the THC 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 3 X 10^-6 M can block NMDA.

To determine the parameter by which THC blocks NMDA, postjunctional membrane (PM) sensitivity was tested by micropersfusion of carbachol with the junctions of THC treated muscle. Application of 20 X 10^-6 M carbachol depolarized the RMP by 23.9 ± 3.7 mV in the control compared with 12.5 ± 2.2 mV in controls. Thus apparently THC does not block NMDA by decreasing the sensitivity of the membrane to cholinergic agonists.

The effect of THC on coupling at the neuromuscular junction is more complex. The neuromuscular junction under study was a normal junction. We have found that THC changes the muscle preparation emulleted in Ringer solution containing 0.9 mg CaCl2 and 4.0 mg MgCl2. The failure rate in cunatal releases was determined using a low frequency train of nerve stimulation ("method of failures"). Perfusion of junctions of these preparations for 15 min with THC 10^-6 M caused complete blockage of cunatal release with 100% failure rate. Thus THC has a substantial effect in reducing the AP evoked release of acetylcholine.


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 (Hartell, Kuffler and Yoshizaki, J. Physiol., 211: 427, 1970; Forteck and Salpeter, J. Cell Biol. 69: 144, 1976; Matthews-Bellinger and Salpeter, J. Physiol., in press, 1978). According to such a model, the 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 ACh site density. Furthermore, if (a) is a significant factor, then the time to peak of a mepc and its amplitude should be positively 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 ACh site density. In 100% of the muscles treated with alpha-Bungarotoxin (α-BTX) and disopropylfluorophosphate (DFP) respectively. 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 peak amplitude) was 31.3 ± 3.2 ms in normal muscle, 220±40 usec after incubation with BTX (10^-10 M for 40 min) and 280±60 usec with BTX plus DFP (10^-8 M for 20 min).

The mean mepc and MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

30 X 10^-6 M  can block NMDA.

1179 ACH RECEPTOR-CHANNELS BEGIN TO OPEN WITHIN 30 µSEC AFTER AGONIST IS APPLIED. Henry A. Lester, Menasche M. Nass*, Mauri E. Hatchett, Dept. of Physiology, and Ahmanson Laboratory of BRI, Sch. Med., UCLA, Los Angeles, CA 90024.

The structural organization of the neuromuscular junction was studied (from 10% to 90% of peak amplitude) was 31.3 ± 3.2 ms in normal muscle, 220±40 usec after incubation with BTX (10^-10 M for 40 min) and 280±60 usec with BTX plus DFP (10^-8 M for 20 min).

The mean mepc and MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

The mean mepc and MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

In the falling phase of these subliminal EPPs. Apparently these junctions function normally, but MEPP frequency was reduced, but MEPP amplitude was either unchanged or increased. Occasionally, MEPP amplitudes were recorded. Thus it was concluded that THC at 30 X 10^-6 M can block NMDA.

As our 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 supramaximal indirectly evoked twitch-tension measurements of frog sciatic/tarsal motor nerves in Ringer's containing 1.5 mM Ca++, 1.5 mM Mg++, 2.5 mM K+, and 114.5 mM Na+. Muscle length was adjusted for max twitch tension. Stimuli were presented at 1.1 per second. The preparations were maintained until the twitch tension decreased by approximately 50%. CSV and CSVP (8.5 to 10 ug/mL in Ringer's) were added to study after stabilization.

Bilateral Standard Ringer's muscle (2.36±.36) mm with max responses within 4 minutes. Indirectly increased twitch tension gradually subsided, reaching control levels in 14 minutes, with total and irreversible block 14 minutes. (Control preparations with no venom were viable for at least 60 minutes). If the muscle chamber was flushed with Ringer's as vehicle response peaked, there was some reversibility of venom action. But wash with Ringer's after short-term reduction of the tension, the facilitation period, time to peak facilitation and time to final block of twitch tension were increased with CSVP containing venom. These effects are consistent with repetitive neural firing caused by venom effects on the Ca++ dependent Na+ conductance system as described by others. However, inability to inhibit the occurrence of block with high Ca++ is not explained by this mechanism.

The presence of three highly purified CSV (Toxin 1, IV, and B140-1) all showed that all toxins facilitated twitch; however, kinetics and appearance of records are different with each toxin. Toxic 1 is a rapid and potent blocker of twitch tension. Toxic 1 showed 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


Song in male zebra finches is part of courtship and agonistic displays. The amount of singing is influenced by testosterone (T): castrate males sing less; but switching male and turtle sexual role reversal increased the amount of singing. Castration (GDx) and T therapy also influence the volume of the syrinx muscle mass. The syrinx is the peripheral organ of song control. As a first step toward understanding mechanisms responsible for effects of T, we hypothesized that T affects neuromuscular transmission or even possibly syrinx muscle itself. Injections of 3H-T lead to heavy concentrations of label over the nucleus of these motoneurons. The preceding observations, from the laboratories of Arnold, Zion, 200, 189, 1. Comp. Neurol. 165 487-76, suggest that T may have an effect on the peripheral organs of song control. As a first step toward understanding mechanisms responsible for effects of T, we hypothesized that T affects neuromuscular transmission or even possibly syrinx muscle itself.

Males and females 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 weight was measured by (Intact = 21.4) g. Replacement for 1 week returned weights to intact levels. Similar changes in larval weight were not found. Activity of acetylcholinesterase (AChE) was measured as specific activity (nmol/min/mg protein) or as total activity (nmol/mg protein/h). AChE activity decreased approximately 40% 2 weeks following GDx. AChE activity in hyoid or pectoralis muscle was not affected.

The possibility of T acting directly in the syrinx as in classical androgen target tissues was examined by measuring for high affinity T binding. Using standard Clark fractionation techniques, 3H-T binding was measured in cytosol prepared from syrinx, larynx, hyoid and pectoralis muscle and in serum triches. In the larynx, high affinity T binding was detected (Kd=0.62nM) with a capacity of 14.8 fmols/mg cytosol protein, values comparable to those in androgen target tissues. T and radioactivity recovered from syringle cytosol was still in the form of 3H-T. No high affinity binding was detected in the other tissues. The specificity of syringeal receptor binding is T > 17β estradiol > 5αDHT > 17β estradiol.

The presence of high affinity T receptors and changes in AChE activity in syrinx after GDx and concentration of label in hypoglossal motoneurons following 3H-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 BM and MB13343 and 5505-RR07065-12 to FN and RF0095.)

1184 ELECTROPHYSIOLOGICAL COMPARISON OF THREE PURIFIED NEUROTOXINS FROM C. SCULPTURATIS VENOM (CSV) AND ISOLATED FROG NEUROMUSCULAR PREPARATIONS. Herbert E. Longenecker, Jr., Gesina L. Longenecker, Barbara Becker, and Barbara Carter. Deps. Physiology & Pharmacology, Univ. of S. Ala. College of Medicine, Mobile, AL 36688.

Injections of scorpion venom into the cervical ganglion of the rabbit sympathetic ganglion. It is interesting to note that even though the data from the sympathetic ganglion (25-27 °C) the fricton, the 140 sec process is potentiation (PTP). This effect of Sr++ on the 140 sec process in the sympathetic ganglion is similar to the effect of Sr on the 460 msec process. This effect of Sr++ is similar to the effect of Ba++ on augmentation at the frog neuromuscular junction. This effect of Ba++ on the 140 sec process in the sympathetic ganglion is similar to the effect of Sr++ on the 460 msec process. This effect of Ba++ on augmentation at the frog neuromuscular junction is similar to the effect of Ba++ on augmentation at the frog neuromuscular junction. This effect of Ba++ on augmentation at the frog neuromuscular junction is supported by NIH grants NS 10277, NS07044, NS05820, NS05363, and a Scottish Rite Fellowship.

SYNAPTIC TRANSMISSION IN THE RABBIT SUPERIOR CERVICAL SYMPATHETIC GANGLION: COMPARISON TO THE FROG NEUROMUSCULAR JUNCTION. K. Lucke, L. Horn, J. A. Zintel, J. C. Eisenberg, and J. McAfee. Department of Physiology, University of Kentucky, Lexington, KY 40506 and 2 City of Hope Med. Center, Duarte, CA 91010.

The preganglionic nerve trunk was conditioned with 1-600 impulses at 5/sec. The EPSP amplitude typically increased during the conditioning train. 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±60 sec (t.s.d.). The addition of Ba++ (0.1-0.2 mM) to the Locke solution caused 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 augmentation at the frog neuromuscular junction. The addition of Sr++ (0.1-0.2 mM) to the Locke solution caused 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 Sr++ on the 17 sec process in the sympathetic ganglion is similar to the effect of Ba++ on augmentation at the frog neuromuscular junction. The addition of Sr++ caused the EPSP to recover with increased Calcium (up to 2mM).
A statistical model indicates that multiple peaks in MEPP amplitude histograms are significant. D. E. Mikkelsen* and M. E. Kriebel (SPON: J. R. Froehlich), Dept. Physiol., Upstate Medical Center, Syracuse, NY 13210.

A statistical model describing the probability density function of MEPP amplitude is 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 mode MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a population 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. 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. Furthermore, when mean MEPP amplitude is decreased, the small mode 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.

A statistical model describing the probability density function of MEPP amplitude is 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 mode MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a population 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. 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. Furthermore, when mean MEPP amplitude is decreased, the small mode 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.

A statistical model describing the probability density function of MEPP amplitude is 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 mode MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a population 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. 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. Furthermore, when mean MEPP amplitude is decreased, the small mode 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.

A statistical model describing the probability density function of MEPP amplitude is 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 mode MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a population 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. 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. Furthermore, when mean MEPP amplitude is decreased, the small mode 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.

A statistical model describing the probability density function of MEPP amplitude is 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 mode MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a population 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. 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. Furthermore, when mean MEPP amplitude is decreased, the small mode 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.

A statistical model describing the probability density function of MEPP amplitude is 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 mode MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a population 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. 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. Furthermore, when mean MEPP amplitude is decreased, the small mode 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.
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. At millimolar concentration it increases MEPP frequency more than 10,000 fold (Heuser et al., Proc. R. Soc. Lond. B. 136: 197, 1952). However, it is difficult to determine to which extent, lanthanum has been shown to have little or no effect in transmission (Harris et al., Nature 268: 265, 1977; Kidoko 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 mV to more than 25 millivolts. The input impedance of these cells was on the average 109 MΩ. If we were 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 buffered Ringer solution, caused roughly a 16-fold increase in MEPP frequency.

To study the effects of lanthanum on newly formed junctions, we recorded MEPPs from junctions 1 to 6 days after nerve-muscle co-culture. MEPPs were continuously monitored in unbuffered Ringer solution, followed by solution containing La3+. 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 buffered Ringer solution containing 100 µM lanthanum. The majority of junctions (11) showed either no increase or less than a 10-fold increase in MEPP frequency in 100 µM lanthanum solution. The remaining junction 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 La3+ develops quickly in Xenopus NMJ cultures compared with rat (Kidoko et al., op cit). However, the developing junction still has a low sensitivity to La3+ compared with the mature junction. Assuming that La3+ interferes with the Ca2+-transmitter secretion coupling, our finding suggests that the early postnatal development of Ca2+-dependent transmitter release mechanism (Supported by USPHS grants NS-10457, NS-08601, F32-NS05631, T32-GMO7211).

**Ruthenium Red Blocks Spontaneous, Evoked, and Ionophore-Induced Release of Transmitter at the Neuromuscular Junction.** J. R. Jackson, B. Pickett, J. Bornstein* and I. Diamond

Ruthenium Red (RR), 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 other Ca-dependent excitation 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 excised microvessels were exposed to 1-5 µM concentrations of crude RR (Sigma). Junctions were additionally exposed at various times to 100 µM concentrations of the Ca ionophore X537A. RR 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 monotonically declining 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 RR, this reduction was not sufficient to cause the disappearance of recordable MEPPs. Simultaneous exposure of junctions to 1 µM RR 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 fiber depolarization and a decline of membrane potential in the region of the endplate. If X537A exposure was delayed until the RR-induced blockade of MEPPs was nearly complete no response to the ionophore was observed except for potential changes of a few mV. These results demonstrate that RR acts to block both extra- and intracellular Ca-binding sites. Intracellular blockade of Ca transients at sites of the nerve terminal or synaptic cleft (Ca) would explain an increase in MEPP frequency as suggested by Aalmaes and Rahamoff (J. Physiol., 248:265) but it does not explain the subsequent block of spontaneous release. RR also blocks the intracellular Ca-binding site required for transmitter release. Extracellular blockade of voltage dependent Ca channels is expected. In the reported specific action of RR, the dye blocks a sequence of reduced evoked release. However, blockade of the effects of X537A action by RR suggests that either the extracellular mechanism of the dye is insufficiently non-specific to prevent interaction of the Ca-ionophore complex with the membrane and that, one or more of the sites to which RR binds are required for Ca translocation at the ionophore site. (Supported by Biochemistry Section, Office of Naval Research, N 00014-77-C-0630, NR 202-091.)

**Δ-AMINOLEVULINIC ACID INHIBITS EVOKED RELEASE BY A PRESYNAPTIC MECHANISM IN RAT NEUROMUSCULAR JUNCTIONS.**

Jack R. Pickett, Joel C. Bornstein* and Iyan Diamond

DIVERSE MECHANISMS OF POSTSYNAPTIC RECEPTOR BLOCKADE AT THE RANA ESCULENTA NEUROMUSCULAR JUNCTION.** D. Fournel-Beenefield and C. A. Quarstel, Dept. of Pharmacology, Faculty of Medicine, The Univ. of British Columbia, Vancouver, B. C., V6T 1U5, Canada.

A variety of drugs act to depress the amplitude of end-plate potentials (EPPs) with receptors for acetylcholine. The mechanisms of this depression can be classified according to the time course of amplitude and time course of miniature end-plate currents (me.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 hexamethonium and curare, me.p.c.s are reduced in amplitude and decay somewhat faster than normal. The time course of me.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 responses of the end-plate to exogenously applied ACh or carbachol is depressed much more than the me.p.c.s; this can be expected from simple models of ACh action with receptor, when binding of ACh to receptor is inhibited.

Local anesthetics and some other agents (e.g., pentobarbital, diphenhydantoin) characteristic ly cause the me.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 anesthetic properties, including at least halothane, pentane and some alcohols (e.g., butanol, pentanol). The effects of X537A action by RuR suggests that either the extracellular mechanism of the dye is insufficiently non-specific to prevent interaction of the Ca-ionophore complex with the membrane or that they inhibit the release of end-plates to exogenously applied cholinergic agonists much less than they depress me.p.c.s. Moreover, the time course of me.p.c.s showed an identical change of amplitude and time course of miniature end-plate potentials by interference with receptor function. Examination of rate constants in models of interaction of these agents with curare or barbiturates. The effects of these agents are difficult to explain, except in terms of modification of channel constants or in rather complicated schemes of infection with receptor. (Supported by grants from the Medical Research Council of Canada and the Muscular Dystrophy Association of Canada.)

Acetylcholinesterase (AChE) accumulates at 70% of nerve-muscle synapses that form in spinal cord explant cultures grown in 50µm curare and 10-100µm dibutyryl cyclic GMP. Enzyme activity was detected histochemically and also by measuring the rate of decay (t) of extracellularly recorded synaptic potentials (ExSPs). Two measures were closely correlated: 76% of synapses with t<1.8ms (30°C) stained for AChE, whereas none with t>2.64ms stained. In addition, the end-plate specific (19S) form of the enzyme was marked by succrose 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 (3x10⁻⁷M), or the AChR antagonist curare (250-500µM) or α-bungarotoxin (2x10⁻⁶M), the mean rates of ExSP decay were prolonged—the percentage of synapses with t<1.8ms 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.

AChE activity increased after denervation of normal and dystrophic muscle. 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. The high AChE activity of dystrophic muscle was accompanied by increased sedimentation (τ) of the 19S AChE form, and by a decrease in the 20S BChE form.

The results reconfirm that AChE rises after denervation of the muscle, and that this regulation is interrupted by dystrophy. The relationship given above to explain F and D is not 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 lability by "empty" vesicles rather than the modification of release probability, 2) that the variations of [Intracellular Ca] plays a major role in the reduction of synaptic transmission caused by dystrophy, and 3) that following a long stimulus, the production of the PTP results from a residue of active calcium that acts both on the amount of available transmitter and on the probability of release.


Channel Open Time Decreases Postnatally in Rat Synaptic Acetylcholine Receptors. S.M. Schuette, C.L. Weiss, and G.D. Fischbach. (Support from the National Institute of Avian Science, UCD, Davis, CA 95616)

The mean channel open time (t) of acetylcholine receptors (AChR's) was studied in skeletal muscle fibers of the chick and rat. AChR channel activities are different, in the developing chick and rat (see below). Acetylcholine receptors (AChR's) were studied in skeletal muscle fibers of the chick and rat. Acetylcholine receptors (AChR's) were studied in skeletal muscle fibers of the chick and rat.

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 AMP, the activity of synapsates decayed ExSPs to a rate of 2-3/sec for 8-12 hours. ExSPs at 5 of 5 previously isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

There are now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

We have now several factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 5µm curare, which reduces the developmental size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Nystatin and colchicine reduced synaptic currents by more than 50% (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 isolated synapsates decayed more rapidly than at 30°C (2.6ms to 1.74ms). This change, in which each synapsate served as its own control, was quite significant.

The effect of quinacrine on neuromuscular transmission was studied on rat soleus muscle using tetrodotoxin (1T) and 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, there was no significant effect on the other rectifying the 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 100 µsec in response to short trains of sural nerve volley of 78 and 62%, respectively. The voltage-current relationship of the EPC became nonlinear in the presence of quinacrine and the time constant of decay phase was less affected than in membrane potential. There was no effect on either the EPC reversal potential or the single exponential function of the falling phase. Quinacrine inhibited acetylcholinesterase (IC50 = 0.3 µM) and the binding of 125I-labelled hydridocholine to the "H-acetylcholine to acetylcholine receptor of the electric organ of Torpedo ocellata (K±=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 U S P H S Grant NS-12063, Med., Baltimore, M D 21201.)


During studies of oxine 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 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 oxime 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 considerab-able NMF still existed and 2) at approximately grade 3 of the AChE staining levels all tetanic contractions were fully sustained. The form of this relationship is in accordance with the 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 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 considerab-able NMF still existed and 2) at approximately grade 3 of the AChE staining levels all tetanic contractions were fully sustained. The form of this relationship is in accordance with the 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 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 considerab-able NMF still existed and 2) at approximately grade 3 of the AChE staining levels all tetanic contractions were fully sustained. The form of this relationship is in accordance with the 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 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 considerab-able NMF still existed and 2) at approximately grade 3 of the AChE staining levels all tetanic contractions were fully sustained. The form of this relationship is in accordance with the 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 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 considerab-able NMF still existed and 2) at approximately grade 3 of the AChE staining levels all tetanic contractions were fully sustained. The form of this relationship is in accordance with the 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 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.
NEURONAL CIRCUITS AND PATTERN GENERATION

The control of the gastric mill network of the spiny lobster by the INN through GABA 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 (PorusMonocerus). 7:215-250.

The gastric rhythm basically consists of synergetic bursts in the E, LG and MG neurons which alternate with the discharge of Int 1. The effect of INN input trains (250 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 neuron 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 intercalated between the two ongoing LG-MG bursts. One may conclude therefore that the responses depend only on the activity status of several elements of the network. This complexity of organization contrasts with the responses of the pyloric network to similar input, where the responses depend only on the activity state of one set of elements.

Repetitive INN 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 intercalated bursts. If the repetitive 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. Silverston.

NEURONAL CIRCUITS AND PATTERN GENERATION


The control of the gastric mill network of the spiny lobster by the INN through GABA 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 (PorusMonocerus). 7:215-250.

The gastric rhythm basically consists of synergetic bursts in the E, LG and MG neurons which alternate with the discharge of Int 1. The effect of INN input trains (250 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 neuron 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 intercalated between the two ongoing LG-MG bursts. One may conclude therefore that the responses depend only on the activity status of several elements of the network. This complexity of organization contrasts with the responses of the pyloric network to similar input, where the responses depend only on the activity state of one set of elements.

Repetitive INN 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 intercalated bursts. If the repetitive 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. Silverston.

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 processing. We have developed a new method for identifying repeating temporal patterns in a single spike train.

The pattern 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 inter-spike interval is independent of all preceding intervals. The patterns detected consist of an arbitrary number of sequential interspike intervals, forming a specific “word.” The patterns can occur at any time in the time series of 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 presentations 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 crustacean claw motor and proprioceptive neurons. Certain patterns occur excessively in these data.

A NEURAL CIRCUIT INVOLVED IN THE CONTROL OF LOCOMOTION IN APELIA. Steven M. Friedman and Behrouz 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 tail 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 body is elevated and attached to the substrate. We have developed a new method for identifying the neural circuit that generates these behaviors hierarchically and posteriorly.

A nearly isolated preparation, consisting of the leech ventral neurons, T, P and N (Nicholls and Baylor, J. Neurophysiol. 31:740-756, 1968; D. Mistick. Thesis. University of California, Davis, 1973) in saline, was used in these experiments. The initiation of crawling requires input from the cerebral ganglia specifying ascending and descending neural segments related to the propodium.

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 & Agra, 1971). The connections of the anterior medial cortex, in the rat, have been studied with silver or horseradish peroxidase (Borgland, 1978). Further studies of the retrograde and anterograde transport of this area, iontophoretic ejections of [3H]-methionine or [3H]-adenosine were made and the tissue was prepared for autoradiography. The rats treated with [3H]-methionine (367-1065 Ci/mole) surviving 1-4 days, and the rats treated with [3H]-adenosine (35 Ci/mole) surviving 3 days. Following a 7-day (methionine) or 21-day (adenosine) exposure period, the tissue was developed, dried flat, stained for Nissl, and examined microscopically. In agreement with previous silver degeneration studies, [3H]-methionine-labelled fibers were found running caudally in the cingulum and in the internal capsule and through and in the dorsomedial caudate nucleus. Fibers left the internal capsule to end in several thalamic nuclei (e.g., dorsal medialis, central medial, ventral medial, parataenial). Further caudally, labelled fibers were found in the medial forebrain bundle, the medial tip of the internal capsule, the lateral septal area, and the ventral superior colliculus. Labelled fibers also ended in the region of the medial substantia nigra and nucleus A10 dopamine-containing cells. Unlabeled cells which did not take up [3H]-methionine also showed label in the brainstem raphe nuclei, in a region medial to the locus coeruleus, and further caudally in the brainstem pyramid tract. Afferent fibres to the caudate, lateral amygdaloid nucleus, and extensive contralateral projections appear similar to the above-mentioned ipsilateral projections. In the [3H]-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. These layers of labeled neurons were much broader than autoradiographic labels. These results further our knowledge on the interconnections of the anterior medial cortex in the rat. (Supported by USPHS grant NS-27574.)
CYCLIC AMP MAY MODULATE PROLONGED ENDOGENOUS BURSTING AND SPIKE BROADENING IN THE VENTRAL WHITE CELL OF FLEUROBRANCHEA CALIFORNICA. MARSHA U. GILLETTE, RANHOR GILLETTE AND WILLIAM J. DAVIS. THIMANN LABS, USC, 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 swimming motor center on behavior of FLEUROBRANCHEA. 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 between cyclic AMP and this conductance was investigated. The temporal, ionic and voltage parameters governing the VWC spike broadening fit a model for progressive spike broadening based on progressive depolarization-induced increase in the rate of Ca++ entry that is characteristic of other calcium channels. Since the VWC is still responsive to additional Ca++ after a 3 hr incubation in 2 mM Ca++ , 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.

The neuronal correlate of swimming recorded from the isolated brain of Tritonia diomedea is a series of alternating bursts in the abdominal nerve cord. The largest of the two relatively large pools of pedal ganglion neurons, the dorsal flexion neurons (DFN) and the ventral flexion neurons (VFN). The neuronal correlate of swimming was 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.
The initiation of normal locomotion. The pleural ganglia appear to provide a relay for ascending input needed for rapid expression. The cerebral ganglion via the C-P is necessary for pedal commissure. When it was sectioned, contractile waves generated 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, with the lesioned side always trailing the intact side. Bilateral sections of the C-PL 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, posterior stimuli to the tail no longer were capable of eliciting rapid aversive locomotion (galloping) and disrupted any ongoing locomotion. Bilateral sectioning of the C-P resulted in more rapid and eliminated rapid locomotion. Although capable of generating waves in the foot, the rate of these waves and locomotion remained essentially normal. A unilaterally stimulated 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 ganglion appears 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 BJR.

Effects of lingual nerve section or neocortical ablations on rat's licking response. Jon D. Kirwin*, Sarah P. Parker*, and David W. Watkins*. (SPON: J. A. Jane). Departments of Neurosurgery and Anatomy, University of Virginia School of Medicine, Charlottesville, Va 22901.

Evans house-bred 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, licking spouts 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 remained essentially constant. After bilateral sectioning of the lingual nerve, licking was abolished, but at less than normal rates (77-89% of pre-op rate), and 3) at less than normal rates (77-89% of pre-op rates). 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 protrusion to contact the spout. Following 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 protrusion, their lick rates were normal. The neocortex plays a role in the rats' licking response, and appears to be related to tongue protrusion and/or the initiation of licking. The sensory inflow from the anterior two-thirds of the tongue (i.e., lingual/chorda tympani) is involved in rhythmic licking, but appears to exert a facilitatory influence on lick rate.

The neuronal basis of the shortening response in leeches. William B. Kristan, Jr., Dept. Biology, University of California, San Diego, La Jolla, CA 92037.

Mild tactile stimulation of a leech produces a localized shortening response which consists of longitudinal muscle contractions limited to a single segment and the bidirectional spread of a single effective contraction. The shortening response spreads to the entire body. The localized shortening response results entirely from the activity of previously identified tactile sensory neurons (Nicholls & Purves, J. Neurophysiol. 31:740-756, 1968), particularly the ventral cord incisures. The activation of these tactile sensory neurons is indistinguishable from the threshold for the shortening response. The hyperpolarization of these cells supports 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 & Stuart, J. Comp. Physiol. 94:121-124, 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, 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 synaptic interactions 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. 209:647-659, 1970) whereas the excitation of the other motor neurons is exclusively polysynaptic. The interneurons responsible for the prolongation of the local contraction are all involved in the generalization of the response to other body segments.

This work was supported by an NSF research grant BNS75-23567.
CENTRAL INTERACTIONS UNDERLYING PATTERN GENERATION FOR ESCAPE SWIMMING IN TRITONIA. Paul R. Lennard, Peter A. Getting and Jacqueline M. Grebmeier*. Dept. of Biological Sciences, 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 inhibitory interneurons (Iain's) (Feldman and Trilovski, Brain Res. 84, 181, 1975) are persistently present during locomotion. Furthermore, these cells comprise a neural circuit such that activation of Renshaw cells via recurrent alpha motor axon collateral would result in persistent post-rest discharges of Iain's and subsequent disinhibition of antagonist alpha motoneurons. RC's and Iain's could therefore contribute to switching between antagonist motoneuron groups during locomotion.

Phase experiments were conducted in isolated brain preparations. Discharge during fictive locomotion was monitored by intracellular recording of the amplitude of short bursts of motoneuron activity. As compared to the conductance to the conductance to the membrane is hyperpolarized during the opposite phase of locomotion. In addition, interneurons, which are rhythmically active during locomotion should be influenced by activation of motoneuron recurrent collaterals. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro. In order to test this model, experiments were performed on cervical spinal cord slices in vitro.
Non-spiking neurons in the cockroach. D.J. Meyer* and B. Walcott

SOCIETY FOR NEUROSCIENCE

or polysynaptic. The membrane potential of type I non-spiking neurons oscillated in phase with motorneuron bursts during rhythmic leg movements. The cells depolarized during extensor bursts and hyperpolarized during flexor bursts. A depolarizing current pulse applied to a type 2 non-spiking neuron during rhythmic leg movements reset the timing of motorneuron bursts. When depolarized by injected current, type 3 non-spiking neurons inhibited the discharge of the slow extensor motorneuron, and had the opposite effect when hyperpolarized. Depolarization of type 2 non-spiking neurons reversibly abolished rhythmic leg movements. These observations are consistent with the hypothesis that the non-spiking neurons are part of the central pattern generator for stepping, however there are alternative interpretations of the data.


Swimmerets are active in a number of different oscillatory behaviors such as righting and larval swimming. The circuitry underlying these behaviors consists of segmental oscillators, which in turn drive swimmeret motor neurons (MW's). Davis and Kennedy (1972) showed that hyperpolarizing a type 2 non-spiking neuron during rhythmic leg movements caused the EPSP's in LM (from P cell inputs) to become tonic for long periods of time, whereas depolarizing the cell reversed this effect. The present study was designed to determine whether the functional connection found is monosynaptic or polysynaptic.

Our results confirm that neurons known from anatomical evidence to be monosynaptic display positive results with the TEA test. For example, injecting TEA into LG(LPG) motorneurons and Interneuron 1 of the gastric system. The P cells which send axons into the STG. P cell inputs to LP had a variable axon pathway, via either the superior- or inferior-cord. The synapses can be discovered using other anatomical and physiological methods. To obtain the results obtained in this system shortly after injection of TEA.

Supported by USPHS Grant NS12295 and the A.P. Sloan Foundation.


P cells" are neurons in the commissural ganglia (CG's) of the lobster CNS which fire in bursts coordinated with the pyloric rhythm. The P cells 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 P's) and monitored the rates at which spikes were generated. The P cell and 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 connections does not affect the interpretation of results obtained in this system shortly after injection of TEA.
NEURONAL SHAPE AND FUNCTION

These experiments were designed to evaluate the immunological, neurological, and histological reactions that take place in the macaque brains following repeated 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 parenchyma. 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 C3 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 neurological symptoms were found in all the noninjected contralateral ventricles. The immunofluorescence approach was localized to the histologically involved 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 in a neurological focus. 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.

A COMPARISON OF SYNAPTIC JUNCTION LOCALIZATION ON LAMPRY SPINAL CORD INTERneURONS 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 lampry spinal cord interneurons following stimulation of a single presynaptic giant axon through the 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. Each was 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 the 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 Ball model. It cannot accurately predict the time course of the synaptic potential. It is concluded from this analysis that functionally identical synaptic junctions may be identified to make synaptic contact at approximately the same electrotonic distance on functionally similar postsynaptic neurites.

In fish, a single neuron—the Mahonter cell (M-cell)—acts as a direct interneuron between sensory input and tail movement. Preferential input also in the tadpole (Raana) and in this cell 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 density-rectified intracellular recording allows the identification 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 II 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 Viliith 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 activa-


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 morphology and alignment and quantification steps sequentially ordered. A continuous gray level T.V. image of the structure(s) being reconstructed (e.g., from the photomicrograph 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. This allows the automatically align and quantitate a number of sections in a series and to match and measure features, examined at higher magnification, such as neuronal somata, axons, dendrites, cell bodies 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.

1231 A MECHANISM FOR THE PRODUCTION OF VERY LOW FREQUENCY REPETITIVE FIRING FROM NERVE ENCODERS UNDER CONSTANT CURRENT STIMULATION. Jurgen Fohleister (SPON: C. A. Terszulo). Laboratory of Neurophysiology, University of Minnesota, Minneapolis, Minnesota 55455.

Several models [1] of neuronal output (e.g., stretch receptor neuron of crayfish) are capable of producing very low impulse frequencies of less than 1 imp/sec when stimuli of very small current are used. Further, the crayfish neuron shows a sizeable nonuni-


Functional properties of neurons of the dorsal lateral geni-

(8


A previous account has been presented on the mechanisms of migration of accessory 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: the axon towards 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 a dependence of the 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 above solutions, and either: a) kept in darkness or exposed to light for adequate periods of time before assessing the position of the pigment in the instantaneous field fixed. 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 complements, the nucleofugal movement of pigment seemed favoured by isotonic CaCl₂ or 15 mM CoCl₂ 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°C) upon nucleofugal migration. Pigment movements in isotonic MgCl₂ were indistinguishable from controls. Oubain 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 optimizing such a process, as high K⁺ and Na⁺ or Li⁺ and Oubain, result in inhibition, whereas those increasing membrane resistance, as Ca⁺² or high external Ca⁺, facilitate the migration towards the DA position. Upon the incidence of light, an influx of Na⁺ which would depolarize the cell, trigger an expansion of the pigment in a process for which Li⁺ could be less suited.

† CONACyT Fellow, México.

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 primary visual cortex, and after, in the spatial analysis of 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. 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* who examined cells after procion yellow injection. We have, for example, been able to perform receptive field and identification experiments on the same identified cell, and to map out the general orientation of the axon. By filling their terminal processes and boutons we were also able to draw a series of horizontal sections which we have used as a basis for determining the alignment of serial sections.

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 neuronal input-output relationships in arbitrary geometry. The model which is used is based on the work of Buts and Cowan (Biophys. J. 12, 661-689, 1974). 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.
A COMPARATIVE STUDY OF VENTROLATERAL AND RECURRENT EXCITATORY POSTSYNAPTIC POTENTIALS IN LARGE PYRAMIDAL TRACT CELLS IN THE CAT. A. Labelle* and H. Deschenes* (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 peduncularis 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)


Phaeochromocytoma 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 thyocytes (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 as a consistent finding in SIDS victims. It is postulated 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.)


In acute cats deeply anesthetized with Nembutal, monosynaptic excitatory postsynaptic potentials (EPSPs) triggered by stimulation of the ventrolateral (VL) thalamic nucleus and the pes peduncularis 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)

SOCIETY FOR NEUROSCIENCE
1241 NEURONAL INTEGRATION CONSIDERED AS A DIRECT FUNCTION. 
Bernard Raciné (Spor: Dalibor Bindra). Dept. Psychol., Univ. de Montréal, Montréal, Québec, Canada, H3C 3J7.

It is shown that the integration of information on the somatodendritic membrane could be considered as a direct function. Formally, a function $f: w_i \rightarrow w_j$ is a direct function, noted $f_d$, iff the result-word $w_j$ is a part of the data-word $w_i$.

$$f_d: w_i \rightarrow w_j$$

where $w_j \in P(w_i)$ and $w_i \in \{ e, i \ldots, eieieiei \ldots \}$ and where the empty word $\epsilon$ corresponds to the lack of stimulation.

From the formal properties of a direct function, it can be shown that on the somatodendritic 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

$$A^* = \{ e, i, ee, ii, \ldots, eiei eieei \ldots \}$$

and where the empty word $\epsilon$ corresponds to the lack of stimulation. Following these considerations, it happens that the grand slow potential issued from spatial and temporal summation is a neuronal word $w_i A^*$ and that this neuronal word $w_i A^*$ is a part of the neuronal word $w_{ij} A^*$ issued from the juxtaposition of the excitatory and inhibitory postsynaptic potentials (e.g. if $w_{ij} \equiv \epsilon e e e e i i$ and $w_i \equiv \epsilon i i e i e i e i$, then $\epsilon e i i e i e i e i e i \epsilon e i e i e i e i \epsilon e i e i e i e i \epsilon e i e i e i e i$).

Thus, it is possible to conceive neuronal integration as a direct function (e.g. $f_d: w_i \rightarrow w_j$ iff $w_j \in P(w_i)$).

From the formal properties of a direct function, it can be shown that the neuron saves at least 300,000 units 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 (1) time economy (II) errors economy and (III) materials economy).

1243 AXON CONDUCTION BLOCK IN NERVE TERMINAL REGIONS CAUSED BY AXON DEPOLARIZATION. Dean Q. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI, 53706.

In the terminal arborization of the excitator axon innervating the open muscle of the crayfish walking leg, action potential propagation falls intermittently during prolonged repetitive stimulation (Katt 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 assessing ATP and arginine phosphate concentrations in the nerve bundles at various times during block development in the excitator axon. After 10 min of stimulation during which conduction failure often occurred, 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-conduction techniques, the diameters of the g-branches at a site of failure were measured. The geometrical ratio, $GR = (d_1^2/2 + d_2^2/2)/d_3^2/2$, where $d_1$, $d_2$, and $d_3$ are the diameters of the two daughter axons and the parent axon, respectively, was calculated at regions of block. The average value was 0.69 (±0.02 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 be extracellular CA+2. Using K+-sensitive microelectrodes, this is being studied currently.

1244 ESTIMATES OF CABLE PARAMETERS FROM AN ANALYSIS OF VOLTAGE TRANSIENTS IN LAMPREY SPINAL CORD NEURONS. William P. Teubl* and Burgess N. Christensen (Spor: 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. Transients were recorded by a second intracellular electrode and analyzed according to the procedure suggested by Jack and Raizada (J. Physiol. 321, 1971) to estimate the cable parameters governing the passive propagation of transmembrane potentials in neurons. For this approach it was assumed that the Ranall model was applicable. This allows reduction of the soma and geometrically complicated dendritic tree to a model, simplistically, consisting of a parallel combination of resistance and capacitance attached in parallel to an equivalent cylinder representing the dendritic tree. Membrane time constant ($\tau_m$), dendritic to soma conductance ratio ($g_t$), and electrotonic length of the equivalent cylinder ($L$) were estimated from the decay 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 Ranall model as an adequate description of the lamprey neurons was provided by computer simulations. Using the estimated cable parameters, a voltage transient was produced from an analytical expression for the Ranall model which describes the voltage recorded at the model soma following a brief current pulse. These simulated transients fit closely with the experimental results even during the early part of the voltage decay. This result suggests that the time constant for the soma and dendritic membranes is similar to that for lamprey neurons. These results have been used to investigate the location of synaptic junctions made by a specific giant axon on the equivalent cylinder.

*Supported by grants NS12151 and NS11669 from the NINCDS, NIH.
NEUROPATHOLOGY
AND
NEUROIMMUNOLOGY
1246 EFFECTS OF PRENATAL EXPOSURE TO THE CHOLINESTERASE INHIBITOR CARBOFURAN ON HATURATION, 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 0.01 or 0.50 mg Carbofuran per kg body weight throughout gestation. Mothers of all experimental groups gave birth to litters of similar numbers of viable, normally developed offspring. Pups born to mothers receiving the higher dose of the carbonate weighed significantly less but by day 28, few if any, differences were noted. Physical maturation and reflex development were evaluated daily in all offspring. Pups born to Carbofuran mothers developed similar 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 Leshley 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 clutch endurance, inclined plane performance or open-field behavior.

The effects were observed 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. Deposits of argentaffin granules were observed in an area extending from the anterior commissure to the anterior olfactory nucleus. No neuropathological changes were observed in offspring of females receiving either 0 or 0.01 mg/kg/day Carbofuran. This research was supported by EPA contract 68-01-1952.

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, Sc.D., University of Virginia School of Medicine, Charlottesville, VA 22901.

Elevated intracranial pressure associated with acute subarachnoid hemorrhage has been attributed to an increase in cerebrospinal fluid (CSF) efflux through arachnoid villi. In an attempt to assess the role of various blood components to acutely induce changes in the blood-brain barrier, in vitro models of CSF-Carbofuran were performed. Normal cultures of avian CSF wereperfused with fetal calf serum and perfused by a pump. No difference was found in the number of viable, overtly normal offspring. Pups born to mothers receiving the higher dose of the carbamate weighed significantly more than control pups. Physical maturation and reflex development were evaluated daily in all offspring. Pups born to Carbofuran mothers developed similar 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 Leshley 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 clutch endurance, inclined plane performance or open-field behavior.

The effects were observed 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. Deposits of argentaffin granules were observed in an area extending from the anterior commissure to the anterior olfactory nucleus. No neuropathological changes were observed in offspring of females receiving either 0 or 0.01 mg/kg/day Carbofuran. This research was supported by EPA contract 68-01-1952.

1248 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, Sc.D., University of Virginia School of Medicine, Charlottesville, VA 22901.

Elevated intracranial pressure associated with acute subarachnoid hemorrhage has been attributed to an increase in cerebrospinal fluid (CSF) efflux through arachnoid villi. In an attempt to assess the role of various blood components to acutely induce changes in the blood-brain barrier, in vitro models of CSF-Carbofuran were performed. Normal cultures of avian CSF were perfused with fetal calf serum and perfused by a pump. No difference was found in the number of viable, overtly normal offspring. Pups born to mothers receiving the higher dose of the carbamate weighed significantly more than control pups. Physical maturation and reflex development were evaluated daily in all offspring. Pups born to Carbofuran mothers developed similar 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 Leshley 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 clutch endurance, inclined plane performance or open-field behavior.

The effects were observed 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. Deposits of argentaffin granules were observed in an area extending from the anterior commissure to the anterior olfactory nucleus. No neuropathological changes were observed in offspring of females receiving either 0 or 0.01 mg/kg/day Carbofuran. This research was supported by EPA contract 68-01-1952.

DECREASE IN LIPID SYNTHESIS IN FIBROBLASTS FROM PATIENTS WITH DYSTONIA MUSCULORUM DEFORMANS. John Blais, Gary E. Gibson & Adriana Vasil*. Neurosurgical Institute, UCA, University of California, San Francisco, CA 94124.

Dystonia musculorum deformans is a neurological disorder which appears to be genetic. However, the biochemical basis of this disorder is unknown. The metabolism of [U-14C]glucose, [1-14C]pyruvate, and [3H]acetate into tissue fibroblasts from dystonia patients was studied. Although one-carbon metabolism in these cultures suggests a possible role of ganglioside metabolism in the mechanism of growth control. (Supported by NIH Grant NS 16P and by The Association for Brain Tumor Research.)

The brain ventricles of 11 species (frog, toad, newt, cong ee, 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 cells involved in traversing the choroid plexi. In this study, 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 Annaus (SPON: A. Goldberg). Dept. Environ. Health, 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 14 days after 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 were killed, corded 0, 5 or 20 week ligate. The control group chloride dissolved corn oil or 50 nM 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 the same. The mean activity level at 14 days was also higher for controls but the difference was not statistically significant. Offspring of mothers treated with 5 mg/kg MeHg 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 first trial failed to meet 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 a 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 CELL INFECTED WITH HERPES SIMPLEX VIRUS. Howard Citelson*, Robert Pozos, Richard Siegel, Paul Lima, Julie Moore* and Steven Oakes*. Deps. 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 10^9 p.f.u. were placed on the culture for 1 hour. After the incubation period of 1 hour, the media was changed. Electrophysiological 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 (plaque). The latter were the predominant response recorded. To detect the plaque 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 electrophysiological observations lasting up to 24 hours did not show the changes observed with virus-infected cultures. Light microscopical observations show that there are no observable cytological changes in the nerve cell bodies at six hours when pronounced electrophysiological changes are observed.

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 sagittal and coronal stained 7 μm brain 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 DAF) hybrid rats on the day of birth.

The grafted lymphoid cells attack the lymphoid tissue of the neonatal host, resulting in a fatal wasting syndrome which was well-developed by postnatal day 14, the day of sacrifice. Unjected littermates served as controls. Total cerebellar area was decreased in GVHD-affected animals by 98% 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 6% 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 cell 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 since layering was preserved. 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).
ACTIVITY LEVEL MEASUREMENTS AS AN INDICATION OF MUSCULAR WEAKNESS IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. L. J. Griffith, J. A. Letterli, N. L. Norcross, M. E. Elderfield, Dept. of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14850, and Inst. 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 accurately determining the extent of muscular weakness in the experimental animal. We have found that measurements of an animal's normal activity can provide an easily useful means of determining the degree of muscular weakness associated with this disease.

Studies were performed in which rats of the Lewis/Ma strain were individually housed in MacLachlan activity cages. EAMG was induced in half of the animals through the inoculation of purified acetylcholine receptor protein (AChR), obtained from Torpedo ocelata, 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 began to increase. Correlations between activity levels and immunological parameters of EAMG will also be discussed.

The results of these studies 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.

(Bolded by the Muscular Dystrophy Association of America)


An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase.

1253 ACTIVITY LEVEL MEASUREMENTS AS AN INDICATION OF MUSCULAR WEAKNESS IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. L. J. Griffith, J. A. Letterli, N. L. Norcross, M. E. Elderfield, Dept. of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14850, and Inst. 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 accurately determining the extent of muscular weakness in the experimental animal. We have found that measurements of an animal's normal activity can provide an easily useful means of determining the degree of muscular weakness associated with this disease.

Studies were performed in which rats of the Lewis/Ma strain were individually housed in MacLachlan activity cages. EAMG was induced in half of the animals through the inoculation of purified acetylcholine receptor protein (AChR), obtained from Torpedo ocelata, 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 began to increase. Correlations between activity levels and immunological parameters of EAMG will also be discussed.

The results of these studies 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.

(Bolded by the Muscular Dystrophy Association of America)


An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase. An inherited neuromuscular disorder of the dog characterized by progressive course in the growing dog. However, the clinical stress has been reported. The disorder is not clinically apparent until 80 DPI at which time the level began to increase.

1254 BIPHASIC EFFECTS OF MAN ACETATE ON THE DEVELOPING BRAIN. R. Maddat, Asma Rabe, Judy Shek, Ruth Dunsm3 and Barbara Tanzer. Neurosciences Laboratory, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Manic-like activity (MLA) and diazepam-induced hypothermia (DIH) are two biologically distinct phenomena that may be useful for comparison with other muscle disorders with similar histochemical changes, including aging changes of muscle. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders. This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparison with similar human disorders.
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 cerebro-vascular, 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 clinico-pathological 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.


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 6 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 above-mentioned regions. In glial cells, these two mitochondrial enzymes remained strong before and after concussion. The alkaline phosphatase (APase) activities reduced in b-wave (7=8 days) and c-wave (3-6 days) amplitudes at 40 days. Other degenerative changes, including fragmentation and cytoplasmic vacuolization, were observed in the retinae of both the pretreatment and control eyes at 40 days after aluminum treatment.


A reproducible tumor model is essential for the study of experimental treatment of tumors. We present a free-hand intracerebral implantation technique, using young rats whose skulls are sufficiently soft so as to permit direct needle puncture, which has advantages of simplicity and rapidity of procedure. Disadvantages include leakage of cells from the skull, large extracranial (EC) masses, intracranial extracerebral (IEC) growth, and intraventricular leakage resulting in spinal cord and brain stem metastases. Studies using spongioblastoma cell line 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.
INFARCTION THRESHOLD IN THE BASAL GANGLIA AND ADJACENT STRUCTURES DETERMINED BY CEREBRAL BLOOD FLOW MEASUREMENT DURING MIDDLE CEREBRAL ARTERY OCCLUSION IN UNANESTHETIZED MONKEYS. Frank M. Marcoux, Richard B. Morawetz, James H. Halsey, Jr., and Umberto DeGirolami. Neuroscience 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. CBF was measured for four weeks following MCA occlusion to determine the precise relation to CBF recording sites. Residual CBF during MCA occlusion was found to correlate closely with the occurrence and character of infarction. When residual CBF was below 12 cc/100 g/min for 2 hours or more, infarction invariably occurred around the electrode tip. The infarction threshold for ischemic durations of less than 2 hours is under investigation and appears to fall off rapidly at around 1 hour.

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/100 g/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 pathophysiology 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.

FATTY ACID COMPOSITION OF HUMAN ERYTHROCYTES IN DUCHENNE MUSCULAR DYSTROPHY. COMPARISON WITH NORMAL AND NEUROMUSCULAR DISEASE CONTROLS. Jack McLaughlin and W. King Engel. J. Neurol. Br., 111:1, 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 190:309, 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 gas 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 many current 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 atrophomorphic lateral sclerosis, myositis, polymyositis, myasthenia congenita, and inherited hypothyroidism). As an example, palmitoleic acid data (area % ± S.D.) were as follows:

<table>
<thead>
<tr>
<th>Normal Controls (N=11)</th>
<th>DMD (N=9)</th>
<th>Definite Carriers of DMD (N=3)</th>
<th>Neuromuscular Disease Controls (N=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.76 ± 0.14</td>
<td>0.81 ± 0.12</td>
<td>0.73 ± 0.08</td>
<td>0.73 ± 0.07</td>
</tr>
<tr>
<td>0.76 ± 0.14</td>
<td>0.81 ± 0.12</td>
<td>0.73 ± 0.08</td>
<td>0.73 ± 0.07</td>
</tr>
</tbody>
</table>

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 ± 0.388 (N=4); DMD, 2.43 ± 0.175 (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.

LOCUTOR EFFECTS OF CATECHOLAMINERGIC DRUGS ON HERPES-INFECTED RODENTS. Richard P. Segal and John F. Watkins. Division of Molecular Laboratories and Research, New York State Department of Health, Albany, NY 12201.

Central nervous system virus infections alter brain catecholamines; yellow fever vaccine virus, West Nile virus, Coxackie virus (Lyecke 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 0.3 ml of a 10–2 dilution of HSV followed at two weeks by an identical intracerebral (IC) inoculation. Baseline activity in this HSV infected, 5.9 ± 105 LH-4/gm. Animals were tested with FP injections of saline and 0.5 and 2.0 mg/kg d-t-ampathetine (an indirect-acting dopamine agonist) immediately following FP and FP-IC HSV and 4 weeks following FP-IC HSV. FP-IC 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 DI depressed both S and DI activity. FP-IC HSV when tested post DI at 6 weeks followed at two weeks by an identical intra-cerebral (IC) inoculation. Baseline activity in this HSV infected stock had a titer of 9.9 ± 105 LD50/ml. Animals were tested with IP injections of saline and 0.5 and 2.0 mg/kg d-t-ampathetine (an indirect-acting dopamine agonist). Immediately following FP and FP-IC HSV mice produced less suppression of DI activity than in controls. These results suggest that chronic HSV HSV reduces DA hypotonicity (characterized by hyperactivity in acute IC only herpes, Lyecke and Roos, J. Neurol. Sci. 22, 1974) and that based on the differential effects of D-t-ampathetine and apomorphine in HSV infected and control mice, the effect may be due to hyperactivity in either the number of, or the sensitivity of the post-synaptic dopaminergic receptors.
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.)
1270 NEUROPATHOLOGY OF "VIBRATOR" - A NEUROLOGICAL MUTATION OF THE MOUSE. William R. Neimar 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 Neurot. Mutants of the Mouse). Initial signs include executional 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.

1271 FOLLOWING FIXATION BY perfusion with 1/2 strength Karnovsky's fixative, brains were embedded in celloidin and sectioned serially at 20 µm. Alternate sections were stained with cresyl violet and by the Loyez method for myelin respectively. The brains of 5 vb/vb 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.

1272 In all vb/vb brains examined, abnormal neurons were a consistent and prominent feature of the following CNS regions: (1) Int. vestibular nucl. ++++; (2) red nucl. +++ (esp. magnocellular div.) (3) large neurons in the brain stem reticul formation ++ (inclusion bodies); (4) the medial, interpositus and lateral deep cerebellar nuclei ++++; (5) mesencephalic reticular formation ++; (6) thalamic reticular nucl. ++; (7) nucl. 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, photomicrographs included large, clear, eosinophilic, and nuclear vacuoles, sometimes to the apparent exclusion of the usual organelles. Larger neurons were particularly prominently affected.

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. Allism 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.
NEUROPEPTIDES
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 125I-β-END, demonstrating an effective range from 4 pM to 10 nM. The sensitivity of the assay is 1-2 femtoles. This antibody exhibits a 10% cross-reactivity with β-END-like immunoreactivity in rat brain has been detected in unextracted samples when compared to blood from hypophysecomized rats. The whole assay and calibration curves are carried out in plasma from hypophysecomized rats. β-END-like immunoreactivity can be detected in normal rat plasma (10 ± 9 femtoles/ml) and exhibits substantial increases with adrenalectomy (1200 ± 24 femtoles/ml). In contrast, samples from 5 healthy normal human males gave significantly lower values when compared to plasma from hypophysecomized humans (12 femtoles ± 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.

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 (Beluzzi & 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. Rate had access to sweetened milk during daily 75-minute sessions. After intakes had stabilized, D-Ala²-D-Leu⁵ enkephalinamide (1, 1 µg), morphine (1 µg) or the β-END-like immunoreactivity by RIA in rat blood: normal levels and comparison to human plasma. Huda Akil, Stanley J. Watson, Jack D. Barchas, and C. R. Lie. 1275

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 125I-β-END, demonstrating an effective range from 4 pM to 10 nM. The sensitivity of the assay is 1-2 femtoles. This antibody exhibits a 10% cross-reactivity with β-END-like immunoreactivity in rat brain has been detected in unextracted samples when compared to blood from hypophysecomized rats. The whole assay and calibration curves are carried out in plasma from hypophysecomized rats. β-END-like immunoreactivity can be detected in normal rat plasma (10 ± 9 femtoles/ml) and exhibits substantial increases with adrenalectomy (1200 ± 24 femtoles/ml). In contrast, samples from 5 healthy normal human males gave significantly lower values when compared to plasma from hypophysecomized humans (12 femtoles ± 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.

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 (Beluzzi & 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. Rate had access to sweetened milk during daily 75-minute sessions. After intakes had stabilized, D-Ala²-D-Leu⁵ enkephalinamide (1, 1 µg), morphine (1 µg) or the β-END-like immunoreactivity by RIA in rat blood: normal levels and comparison to human plasma. Huda Akil, Stanley J. Watson, Jack D. Barchas, and C. R. Lie. 1275

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 125I-β-END, demonstrating an effective range from 4 pM to 10 nM. The sensitivity of the assay is 1-2 femtoles. This antibody exhibits a 10% cross-reactivity with β-END-like immunoreactivity in rat brain has been detected in unextracted samples when compared to blood from hypophysecomized rats. The whole assay and calibration curves are carried out in plasma from hypophysecomized rats. β-END-like immunoreactivity can be detected in normal rat plasma (10 ± 9 femtoles/ml) and exhibits substantial increases with adrenalectomy (1200 ± 24 femtoles/ml). In contrast, samples from 5 healthy normal human males gave significantly lower values when compared to plasma from hypophysecomized humans (12 femtoles ± 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.
HIGH AFFINITY BINDING SITES FOR THYROTROPIN RELEASING HORMONE IN BRAIN AND RETINA: RESEMBLANCE TO PITUITARY RECEPTORS. David B. Burt, Dept. Pharmacology & Exp. Ther., Sch. Med., University of Maryland, Baltimore, MD 21201.

Thyrotropin releasing hormone (TRH) binds with high affinity (Kd < 30 nM) to the anterior pituitary which appears to represent its physiological receptors. Similar sites were previously detected in rat brain (Burt and Snyder, Brain Res. 359:309, 1985), 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 ([3H]TRH (115 Ci/mmol) with the more potent and specific 3-methylhistidyl TRH and the TRH content (Schaeffer et al., Proc. Nat. Acad. Sci., 74:3579, 1975). The retina has been found to possess a similar high level of high affinity binding, in interesting agreement with previous reports of its high-affinity TRH content (Schaef er et al., J. Neurochem., 32:1575, 1979). Both nucleus accumbens and retina binding sites resemble pituitary receptors in their affinities for TRH and for 15 structur al analogs of TRH (115 Ci/mmol) with the more potent and specific 3-methylhistidyl TRH (KD = 10^-30 nM) to sites in the anterior pituitary which appear to be of low affinity binding sites. Under these conditions no breakdown of [3H]TRH was detected. Bound radioactivity was separated by filtration. High affinity binding was over half of the total binding in the results discussed below.

All 3 classes of sites appear to have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites receptors in their affinities for TRH and for 15 structural analogs of TRH appear to have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites have the same kinetics of association and dissociation. Other (generously supplied by Abbott Laboratories). All 3 classes of sites have the same kinetics of association and dissociation.
Recent investigations indicate that neuroactive peptides may play both mediating 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 induces a 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 (Bhril 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 $^{32}$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 radiophosphorus into one group of specific proteins (MW 15-20K). The selective inhibition of phosphorylation by met-enk was found to be dependent on time and concentration of the peptide. In a different fashion, the inhibition of the phosphorylation of these proteins 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 stimulation of the phosphorylation of specific proteins. Supported by intramural funds from the No. Inst. of Psychiatry to E. G. Brunngraber.
SOCIETY FOR NEUROSCIENCE

1285 MORPHINE OR STRESS INDUCED INCREASES OF PLASMA ß-ENDORPHIN AND PROLACTIN ARE PREVENTED BY DEXAMETHASONE PRETREATMENT. Edward D. Franzch, Floyd E. Bloom, Catherine Rivier, Roger Guillemin, Jean Rossier, A.V. Davis Center for Neurobiology, The Salk Institute, La Jolla, CA 92037.

Slight condition of stress, plasma levels of ß-endorphin (B-E) and ACTH are concomitantly increased. Opiates are also known to alter anterior pituitary function and to increase plasma levels of corticosterone. We have studied by hypothalamic mechanism. Rats receiving morphine sulfate (MS) 20 mg/kg s.c. showed a 9-fold increase in B-E immunoreactive material like material 1 hr after injection and a return to control values by 24 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) (0.1 mg/kg at 2 hr before and 1 hr 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 the 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. These results subjected 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.T. 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. Grud, L. M. Huang, J. L. Barker, and T. G. Smith*, LMP, 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 membrane potential recording techniques and extracellular iontophoresis of ENK and NAL. When applied to the neuronal soma, ENK frequently produced dose-dependent membrane depolarizations which were not 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 blocked by naloxone. A 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. Occasionally 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 also blocked the response to ACh and glutamate. Muscimol and bicuculline were remarkably specific in antagonizing the transmitter-like 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.


In the LC of the rat, SP is localized within boutons that make axodendritic synapses with the noradrenaline-containing neurons. The electrophysiological 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 the antagonist, baclofen (bcl), 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 theanine cyclic peptide (C-11 octapeptide) of SP and by tachykinins with SP-like activity (elidiosin and physalaemin). By contrast, TRH, bradykinin and neurotensin were ineffective even at high concentrations. SP excited the LC 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 (1 mM, pH 4.5) from ionophoretic pipettes was monitored "in vitro" using a sensitive radiolmmunoassay. Its time course varied from 1.6 x 10^-2 in the presence of 17.5 mM Na+ to 0.08 x 10^-2 in the presence of 170 mM Na+. The effect of a retaining current on the firing rate of LC neurons was studied using intracellular recording techniques and extracellular iontophoresis of ENK and NAL. When applied to the neuronal soma, ENK frequently produced dose-dependent membrane depolarizations which were not 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 blocked by naloxone. A 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. Occasionally 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 also blocked the response to ACh and glutamate. Muscimol and bicuculline were remarkably specific in antagonizing the transmitter-like 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.


Leucine enkephalin was shown 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 rat ventricle. Neurons in the LC were recorded to determine the effect of ICV administration of ENK- and saline-injected rats. Seventeen to 18 days after surgery, groups of 6-9 rats were injected ICV with either 10 ng saline, 12.5, 25, 50 or 100 ng D-Ala2-Met5-enkephalamide (ENK). Unanesthetized 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.

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 chamber 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 2.5 ul saline and 16 with 2.5 ul 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. Initial levels 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.
Dissociation of Effects of Somatostatin on Turnover of Catecholamines in the CNS. Viktor Havlicek, Robert Herchi and Milan Buzek, Dept. of Physiology, U. of Manitoba, Winnipeg, Canada.

Somatostatin (SRIF) administered into rats intracerebroventricularly (ICV) or into various brain regions (neostriatum, amygdala, hippocampus) supraor cortically (s.c.) causes a graded excitatory response: animals begin running in circles; grooming activity may be replaced by excessive and stereotyped scratching, and finally become fulminant. These changes do not appear, particularly in rats, with enkephalin peptides. Intracerebroventricularly (i.c.v.) administered SRIF (1-100nmol) induces, in rats, subcortically generated paroxysmal waves and cortical seizures. These changes do not appear, particularly in other dopamine agonistic effects, such as analgesia or behavioral immobility and rigidity. Similar electroencephalographic (EEG) abnormalities are seen in mice, with guinea pigs and squirrel monkeys after SRIF administration. Intravenous injection of SRIF (20-26ng/kg) in albino mice, but not in rats, produces paroxysmal activity, analgesia and rigidity. In an attempt to anaesthetize these animals, we found that these changes were potentiated in either the generation or elaboration of this phenomenon, radio frequency lesions have been made in a variety of sub-cortical loci in rats in order to test the effects on EEG and spontaneous locomotor activity. Somatostatin administered centrally in the forebrain and cerebellum. The fluorescence method for the measurement of DA and NE was used. Results are presented in table 1.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Treatment</th>
<th>NE ng/g</th>
<th>P</th>
<th>DA ng/g</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forebrain</td>
<td>sal &amp; sal</td>
<td>24</td>
<td>+ 0.003</td>
<td>0.035 + 0.028</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>α-MT &amp; sal</td>
<td>28</td>
<td>+ 0.003</td>
<td>0.018 + 0.022</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>α-MT &amp; SRIF</td>
<td>20</td>
<td>+ 0.003</td>
<td>0.063 + 0.006</td>
<td>0.001</td>
</tr>
<tr>
<td>Hindbrain</td>
<td>sal &amp; sal</td>
<td>10</td>
<td>+ 0.003</td>
<td>0.013 + 0.002</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>α-MT &amp; sal</td>
<td>10</td>
<td>+ 0.003</td>
<td>0.010 + 0.002</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>α-MT &amp; SRIF</td>
<td>18</td>
<td>+ 0.003</td>
<td>0.002 + 0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>sal &amp; sal</td>
<td>19</td>
<td>+ 0.003</td>
<td>0.019 + 0.003</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>α-MT &amp; sal</td>
<td>17</td>
<td>+ 0.003</td>
<td>0.010 + 0.002</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>α-MT &amp; SRIF</td>
<td>16</td>
<td>+ 0.003</td>
<td>0.002 + 0.002</td>
<td>0.001</td>
</tr>
</tbody>
</table>

α- and β-methyl-P-tyrosine (α-MT 250ng/kg) and (b) 25 min after α-MT injection. Controls were injected with saline or α-MT i.p. and with saline i.c.v. Rats were sacrificed by decapitation 60 min after receiving α-MT. The brain was rapidly dissected into three regions: forebrain, hindbrain and cerebellum. Pre- and post-treatment with SRIF significantly altered the metabolism of NE and DA. Similar changes for NE were seen for hindbrain, while these changes potentiating the effect on NE and inhibiting the excitatory response: animals begin running in circles; grooming activity may be replaced by excessive and stereotyped scratching, and finally become fulminant. These changes do not appear, particularly in rats, with enkephalin peptides. Intracerebroventricularly (i.c.v.) administered SRIF (1-100nmol) induces, in rats, subcortically generated paroxysmal waves and cortical seizures.


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 use the ECT to produce a generalized seizure, may involve a functional release of endorphins.

Male Wistar rats (Walter Reed Strain) weighing 250-300g were affixed with wing clips on the pinna of each ear to serve as electrodes for transauricular ECS. Naloxone (10mg/Kg) or saline were injected intraperitoneally in a volume of 0.1ml/100g body weight. 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 [Chueh 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.

If ECS produced 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 only partially induced by electroconvulsively-induced catalepsy. Naloxone preinjection significantly diminished this ECS-induced cataleptic state. Tail-flick latencies were elevated following ECS treatment, an effect which 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 108mmHg to 228mmHg within the first second of electroshock. Naloxone was without effect on this hypertensive response. Administration of saline and naloxone treated rats following this surge demonstrated significant effects of naloxone on blood pressure and heart rate which persisted during the following 35 seconds after shock.

Our data suggest that endorphins are released during ECS in rats and that they play a role in post-ECT behavior. We suggest that a release of endorphins play a role in the therapeutic effects or complications of ECT in man.
were implanted in the posterior limb of the internal capsule; stimulation of this area produced no increase in ventricular levels of \( \beta \)-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 \( \beta \)-endorphin-like substances. Intraventricular administration of human \( \beta \)-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 \( \beta \)-endorphin-like substances in humans and suggest that stimulation may have produced the release of the substances.


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 immunofluorescence. 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 \( \mu \) 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. Somatodendritic cell bodies in the lateral and medial clusters occur in the periventricular and dorsomedial nuclei of the hypothalamus. Varicose fibers run dorsally and laterally from these two hypothalamic clusters. Caudoventrally, 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 ventrally from this cluster in the substantia nigra. A second 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. is recipient of fellowship from FAPESP, Brazil.


Electrical stimulation of the periaqueductal and peri-ventricular gray matter provides significant relief from severe pain and other emotional disturbances, 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 \( \beta \)-endorphin-like substances were determined in each sample by a radioimmunoassay. This technique may also detect molecules larger than \( \beta \)-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 surgical 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 \( \beta \)-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 \( \beta \)-endorphin-like substances.

**EREKPHALIN MODULATION OF AMINO ACID RESPONSES ON CULTURED SPINAL NEURONS.** L.M. Huang*, D.L. Cruol, J.L. Barker and T.G. Smith* (SPON: R.G. Wagner) LMP, Hines, IL, Chicago, IL 60614.

The effects of the opiate peptide leucine-enkephalin (ENK) and its antagonist naloxone (NAL) on amino acid responses of cultured fetal mouse spinal cord neurons were studied using intracellular recordings of membrane potential and current made with current and voltage clamp techniques. The putative inhibitory amino acid glycine (GA) inhibited action (AC) potential and quisqualate (Q) responses of 19 neurons, were blocked by the ENK and NAL, but were not blocked by the glycine antagonist. These results indicate the ability to identify the inhibitory influence on the excitatory effects of amino acid responses of cultured spinal cord neurons by selective blockade of ENK and NAL, but not glycine, antagonists. This model has demonstrated the potential for the study of the functional role of ENK and NAL in the inhibition of amino acid responses of cultured spinal cord neurons.

**"OPIOD" BEHAVIORAL EFFECTS FOLLOWING ADRENOCORTICOTROPIN OR \( \beta \)-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. 10005.

Microinjection of adrenocorticotropic (ACTH 1-24) in the periaqueductal gray (PAG) of rats resulted in a dose-dependent hyperactivity characterized by repeated and rapid high leaps ("flying") similar to the behavior seen in opiate dependence. 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.

We previously reported (Jacquet and Lajtha, Science, 1974) that microinjection of morphine in the PAG resulted in 2 "paradoxical" effects: (1) analgesia/catalepsy, and (2) hyper-reactivity, characterized by rapid and repeated high leaps. Pretreatment with naloxone blocked (1) but not (2). Injections of NAL in the PAG resulted only in (1) but not (2). Injections of morphine in the PAG resulted in (1) and (2). Injections of naloxone blocked (1) and (2). The present studies suggest that morphine in the PAG may function as a powerful analgesic, but may also have a role in the production of a "paradoxical" effect, i.e., analgesia/catalepsy, which was not stereospecific for opioids and was naloxone-insensitive. While injections of the unnatural (+)-morphine in the PAG resulted only in (1) and (2) (Jacquet et al, Science, 1977). These observations suggest that morphine effects in the PAG were mediated by 2 classes of receptors, one being stereospecific for opiates and naloxone-sensitive, of which the natural enantiomer was the agonist, and the opposite which was not stereospecific for opioids and was naloxone-insensitive. Our present evidence suggests that ACTH may be the endogenous ligand of the second class of receptors.

Systemic injections of morphine never resulted in "flying." Moreover, pretreatment of rats with systemically-administered morphine blocked "flying" following microinjection of morphine in PAG. Naloxone did not affect this behavioral response. These differential effects of morphine following systemic or local administrations suggest that following systemic administration, morphine acts by producing an excitatory effect on the CNS, acting by 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 weakened by tolerance development, of the inhibitory influence exerted by the endorphin receptor.

Intrahypothalamicly injected β-endorphin evokes hypothermia in the rat (Bloom et al., Science, 1976; 194: 209-20). The site of this action could be a hypothalamic site which parallels that of the release of the vasopressin in the preoptic/anterior hypothalamus (POAH), but the LCY route of administration does not preclude the involvement of other structures which function in the vasoressin release. Hence, we measured the rectal temperature (T) after the injection of β-endorphin into the POAH.

24 μg guide cannula was implanted above a POAH or LCY site in 34 male Sprague-Dawley rats. POAH and LCY injections were made via a 30 μg injectors over a 30 sec interval. In the volume of injection, 0.3 or 1.1 μl in the POAH and 0.1 μl in the LCY. Special CSF vehicle or β-endorphin, in doses of 7.4, 1.48 and 3.7 nmole (nM) was microinjected in the POAH (n=6), whereas the LCY dose was 3.7 and 7.4 μM (μM) measured by monitoring a thermistor in the rectum of the unrestrained rat.

β-Endorphin evoked a hypo- or hyperthermia depending on the dosages route of administration. Comparing the increase in T in 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 nmole of β-endorphin caused a significant but nonseose-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 LCY, on the other hand, 0.74 and 3.7 nmole of β-endorphin caused no significant change in T (MP = 37.7 ± 0.6, 38.1 ± 0.5°C). The LCV injection of 7.4 μM 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 Indom (10 mg/kg), slightly attenuated the drop in T seen after β-endorphin 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 hypothermia 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 LCY injection of β-endorphin, the drop in T may not reflect an endogenous immunoregulation 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 hypothermic action is, at least partly, mediated by prostaglandins.


Injected into the lateral cerebral ventricle (LCV), β-endorphin evokes hypothermia in the rat (Bloom et al., Science, 1976; 194: 209-20). The site of this action could be a hypothalamic site which parallels that of the release of the vasopressin in the preoptic/anterior hypothalamus (POAH), but the LCY route of administration does not preclude the involvement of other structures which function in the vasopressin release. Hence, we measured rectal temperature (T) after the injection of β-endorphin into the POAH.

24 μg guide cannula was implanted above a POAH or LCY site in 34 male Sprague-Dawley rats. POAH and LCV injections were made via a 30 μg injectors over a 30 sec interval. In the volume of injection, 0.3 or 1.1 μl in the POAH and 0.1 μl in the LCY. Special CSF vehicle or β-endorphin, in doses of 7.4, 1.48 and 3.7 nmole (nM) was microinjected in the POAH (n=6), whereas the LCY dose was 3.7 and 7.4 μM (μM) measured by monitoring a thermistor in the rectum of the unrestrained rat.

β-Endorphin evoked a hypo- or hyperthermia depending on the dosages route of administration. Comparing the increase in T in 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 nmole of β-endorphin caused a significant but nonseose-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 LCY, on the other hand, 0.74 and 3.7 nmole of β-endorphin caused no significant change in T (MP = 37.7 ± 0.6, 38.1 ± 0.5°C). The LCV injection of 7.4 μM 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 Indom (10 mg/kg), slightly attenuated the drop in T seen after β-endorphin 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 hypothermia 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 an endogenous immunoregulation 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 hypothermic action is, at least partly, mediated by prostaglandins.


Aging is accompanied by a decreased adaptability of some homeostatic mechanisms under hypothalamic regulation. One such function, water balance, is controlled by vasopressin. This magnumcellular 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 vasopressin (BVP), human estrogen-stimulated neurophysia (ESN), and human nicotine-stimulated neurophysia (NSN). Sections adjacent to those stained for neurophysia were examined for CA fluorescence and the degree of CA varicosity/vasopressin 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 bellow BVP (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 anti-sera, especially in the processes of the hypothalamo-neurohypophysial 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 neurophysia (ESN, NSN,BPN) containing perikary with apparent CA contacts were comparable in both ages. These data indicate that the amount of immunoreactive neurophysia decreased with aging and that the use of the more specific anti-sera focus on a possible alteration in vasopressin content. Whether this represents an alteration in synthesis, storage or release of neurophysia is unknown. We thank Earl A. Zimmerman for the antisera and Douglas M. Bowen for the animals. Supported by NS 11642 (JRS).

EXCITATORY ACTION OF NEUROTENSIN ON CAT DORSAL HORN NEURONES IN LAMINAE I-III. V. Mikolaj Miletic and Mirjana Randic, Iowa State University. (Supported by PHS Grant NS 12972-01 NSF Grant BNS 23871 and Salsbury Foundation.)
CHARACTERIZATION OF THE BOMBESIN RECEPTOR IN MAMMALIAN BRAIN.  
Terry U. Moody* and Candace B. Pratt (SPON: W. E. Bunsey, 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 anologue of bombesin binds specifically to rat brain membranes with high affinity (Kd = 1 nM). The high affinity binding was noncooperative and saturable, with an estimated 3 moles of sites/g wet tissue. The association and dissociation rate constants were 1.1 x 10^6 M^-1 sec^-1 and 1.0 x 10^-1 min^-1 respectively. Subcellular fractionation studies revealed that the density of sites is 3-fold greater in synaptic than nuclear or mitochondrical 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-bombesin with greater affinity than do those analogues which possess lower biological activity. In particular, numerous amino acid analogues, di- and tripeptides, and larger peptides, appear to be essential for interaction with the bombesin receptor whereas numerous amino acid residues near the NH2 terminal, e.g., Lys or Leu, are not essential. Other putative neurotransmitters and brain receptor antagonists are not competitive for the high affinity tyrosine-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.

EFFECTS OF PHARMACOLOGICAL AND ENDOCRINOLOGICAL MANIPULATION ON NEUROTENSIN-INDUCED HYPOTHERMIA.  

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 (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. Parachlorophenylalanine (PCPA) and 6-hydroxydopamine (6OHDA) were utilized to deplete brain serotonin and catecholamines respectively and these depletions were confirmed by radioenzymatic assays. Furthermore, IC NT injection into thyroidectomized rats, but not control rats, resulted in a significant reduction in the characteristically high levels of serum immunoreactive TRH observed in TX animals. These results demonstrate that two endogenous neurotransmitters, NT and TRH, appear to be antagonists in certain systems. (Supported by NICHD HD-03110, HD-10570, and NINH grants MH-15631, MH-00013, and MH-22536).

ACCUMULATION OF 3H-HYDROTROPIN RELEASING HORMONE (TRH) BY RAT CEREBELLM SLICES.  
P. A. Begg, J. M. Parry, B. S. Gordon, M. F. O'Donnell, Dept. of Cell Biology and Biochemistry, Univ. Texas M. HSC. Ctr., Dallas, TX 75235.

The extrahypothalamic distribution of TRH (Endocrinology 95: 509-514, 1974) suggests a widespread central action for this NT-like peptide in addition to its hypothypofusitive function. The current work was done to investigate the possible existence of a transport mechanism for TRH in extrahypothalamic brain tissue. Some studies have suggested that TRH is transported in neuronal processes which might be related to its synaptic actions. Two hundred µm sagittal slices of rat cerebellum were incubated with [3H]-TRH (100 µΜ, 5 x 10^4 cpm) with or without 10 µΜ TRH to prevent its degradation (Biochem. and Biophys. Res. Com. 73: 507, 1976). Time course studies showed a rapid accumulation of [3H]-TRH in slices incubated in 25°C or 37°C. Maximum uptake occurred at 60 min of incubation. The process responsible for [3H]-TRH uptake had many of the properties of a high affinity transport system: 1) it was temperature sensitive (Q10 = 1.48); 2) it showed saturation kinetics; and, 3) tissue:medium ratios of 5:1 were attained after 60 min incubation at 37°C. Bicucullin in the incubation medium increased the uptake of label by 38%; however, chromatographic analysis revealed that in these conditions 30% of the total dpm were TRH metabolites and 70% were [3H]-TRH. This indicates that Bicucullin 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 be labeled in both striatal and collateral inputs to Purkinje cells (Nature 255: 233, 1978; 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 00531-01 (J. F. M. ), and NSF grant BNS 77-01174 (D. J. W.).

ACCUMULATION OF 3H-HYDROTROPIN RELEASING HORMONE (TRH) BY RAT CEREBELLM SLICES.  
P. A. Begg, J. M. Parry, B. S. Gordon, M. F. O'Donnell, Dept. of Cell Biology and Biochemistry, Univ. Texas M. HSC. Ctr., Dallas, TX 75235.

The extrahypothalamic distribution of TRH (Endocrinology 95: 509-514, 1974) suggests a widespread central action for this NT-like peptide in addition to its hypothypofusitive function. The current work was done to investigate the possible existence of a transport mechanism for TRH in extrahypothalamic brain tissue. Some studies have suggested that TRH is transported in neuronal processes which might be related to its synaptic actions. Two hundred µm sagittal slices of rat cerebellum were incubated with [3H]-TRH (100 µΜ, 5 x 10^4 cpm) with or without 10 µΜ TRH to prevent its degradation (Biochem. and Biophys. Res. Com. 73: 507, 1976). Time course studies showed a rapid accumulation of [3H]-TRH in slices incubated in 25°C or 37°C. Maximum uptake occurred at 60 min of incubation. The process responsible for [3H]-TRH uptake had many of the properties of a high affinity transport system: 1) it was temperature sensitive (Q10 = 1.48); 2) it showed saturation kinetics; and, 3) tissue:medium ratios of 5:1 were attained after 60 min incubation at 37°C. Bicucullin in the incubation medium increased the uptake of label by 38%; however, chromatographic analysis revealed that in these conditions 30% of the total dpm were TRH metabolites and 70% were [3H]-TRH. This indicates that Bicucullin 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 be labeled in both striatal and collateral inputs to Purkinje cells (Nature 255: 233, 1978; 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 00531-01 (J. F. M. ), and NSF grant BNS 77-01174 (D. J. W.).

We have previously found that subcutaneous injection of cyclo (Leu-Gly) into the C-term fragment of oxytocin protected against pururomycin amnesia when given immediately after or 24 hr before Y-maze training. Cyclo (Leu-
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, forebrain, hippocampus + entorhinal cortex, diencephalon, basal ganglia and cerebrum 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 extracellular space to that calculated for the extracellular space was greater than 1 by 7 hr days after injection in brain and plasma. The ratio of the concentration of the peptide in the extracellular space to that calculated for the extracellular space was greater than 1 by 7 hr.

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 pururomycin-induced amnesia (Rm=92, P < 0.005). 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 10399 and NSF Grants GB 42753 and BNS 76-11779.)

1306 ENKEPHALIN ACTS TO INHIBIT LOCUS COERULEUS MEDIATED BEHAVIORS. D.E. Redmond, Jr., N.S. Gold and Y.H. Huang, Dept. of Psychiatry, Yale University, New Haven, Connecticut 06510.

Both intravenously1 and iontophotically2 administered morphine diminishes the neuronal activity of the noradrenergic nucleus locus coeruleus, an effect directly mediated by opiate receptors, as shown by naloxone blockade.1,2 Similar effects of iontophotically or intravenously administered α-adrenergic agonists are specifically blocked by low doses of the α-adrenergic antagonist piperazine.3 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 Macac arctoides were compared after morphine, naloxone, piperazine, or a parenteraly active synthetic pentapeptide (Sandos FK 33824) were infused intravenously from outside a sound dampened isolation chamber. Piperazine (1.0 mg/kg) increases behaviors indistinguishably from the effects of human threats, or of low intensity electrical stimulation of the locus coeruleus.5 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 FK 33824 (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 situations, 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.4


1307 NEUROHYPOTHALAMIC PEPTIDES, NOREPINEPHRINE AND ETHANOL TOLERANCE. R. F. Ritzmann, Paula L. Hoffman*, and Boris Tabakoff, Dept. of Physiology & Biophysics, University of Illinois Medical Center, Chicago, Illinois 60680.

The functional tolerance which develops during chronic ethanol consumption has been postulated to resemble other adaptive phenom-
ena such as the acquisition of a learned response or memory (Taba-
koff 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. Neurohypophysyal 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, the functional 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 these mice were terminated 9 days after withdrawal, tolerance subsequently disappeared with its normal time course of approximately 6 days. In a series of experiments, mice were exposed 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. As expected, these mice showed a prolongation of the duration of tolerance in the 6-OHDA-injected ethanol-fed mice. Tolerance dissipated with a similar time course in AVP- and saline-injected mice i.e. within 5 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.


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 1(No. 8070):929,1978). 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. Neurohypophysyal 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, the functional 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 these mice were terminated 9 days after withdrawal, tolerance subsequently disappeared with its normal time course of approximately 6 days. In a series of experiments, mice were exposed 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. As expected, these mice showed a prolongation of the duration of tolerance in the 6-OHDA-injected ethanol-fed mice. Tolerance dissipated with a similar time course in AVP- and saline-injected mice i.e. within 5 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.
ENKEPHALIN FIBERS ARE PRESENT IN THE Pars nervosa OF THE Rat pituitary. Dehydration decreases markedly their enkephalin content. Jeunet and colleagues (1982) have measured L-e in the hypothalamus and pituitary and found 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 pretreatment with naloxone sensitizes the rat pituitary. B-E was concentrated in pars distalis and pars intermedia and was absent from pars nervosa. L-e was mostly in pars intermedia, L-e was found mostly in pars nervosa.

Total content Per cent per pars

<table>
<thead>
<tr>
<th></th>
<th>Whole pituitary</th>
<th>Pars distalis</th>
<th>Pars intermedia</th>
<th>Pars nervosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-e i.l.*</td>
<td>1.3 µg</td>
<td>60%</td>
<td>35%</td>
<td>5%</td>
</tr>
<tr>
<td>α-MSH i.l.</td>
<td>450 ng</td>
<td>20%</td>
<td>68%</td>
<td>12%</td>
</tr>
<tr>
<td>L-e i.l.</td>
<td>9%</td>
<td>13%</td>
<td>78%</td>
<td></td>
</tr>
</tbody>
</table>

L-e i.l. immunoassayable like material.

By immunocytochemistry, fibers visualized with L-e antiserum were seen in pars intermedia and pars nervosa. The L-e immunoreactivity in the pars intermedia extracts was coeluted with synthetic L-e on gel filtration column. Neither vasopressin (AVP and LVP) nor oxytocin cross-react with our L-e assay at doses up to 100 µg/assay tube. Our assay shows a 32% cross-reactivity with Met-5 enkephalin, which may thus be a part of L-e i.l. I.

After dehydration (NaCl 22 drinking fluid for 5 days) L-e levels in the posterior lobe of the rat pituitary were 37% of the control animals while no changes were noticed for B-E. Enkephalinergic bodies were visualized by immunocytochemistry in the paraventricular nucleus of the hypothalamus after cocaine pretreatment. Although we have measured L-e in the hypothalamus there were no changes after dehydration. These results indicate that enkephalinergic fibers are located there to modify 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 possibly have an excitatory role in the regulation of vasopressin secretion.

Supported by DA 07850-02; J.R. is supported by INSERM (France).


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 Oral Research, L. B. Ashworth, 1978). An apparent exception is the hippocampal region where, in the stratum radiatum, both centripetal and centrifugal activity is increased by opiates. These data indicate that enkephalinergic fibers from the hypothalamus may be acting on HPN to hyperpolarize their activity. This takes place in the stratum radiatum and is blocked by bicuculline.

HPN's) were excited while cells with non-bursting spontaneous activity (probable interneurons) were only inhibited. Employing barrel iontophoretic pipettes, or with multi-barrels glued to one tip, we were able to excite HPN's via lateral HPN's with tip separations of 30-60 µm. When B-E i.l. 750 pg 9% 13% 78%

B-E i.l.* 1.3 µg 60% 35% 5%

ow = -MSH was mostly in pars nervosa, α-MSH was mostly in pars intermedia, L-e was found mostly in pars nervosa.

These results suggest that the naloxone-sensitive opiate receptors in the HPN region depends upon the local circuitry. This excitatory mechanism is produced by intracerebroventricular opiates (Henriksen et al., Proc. Soc. Neurosci. 3:291, 1977). To investigate the mechanism of this excitatory response, HPN's were tested with conventional five-barrel microiontophoresis and with paired 3-barrel iontophoretic pipettes, or with multi-barrels glued to one tip, we were able to excite HPN's via lateral HPN's via recurrent activation of basket cells, thought to release GABA onto HPN's. This basket cell-evoked inhibition was shown to be mediated by GABA receptors, since it was blocked by bicuculline, and was absent from pars nervosa, α-MSH was mostly in pars intermedia, L-e was found mostly in pars nervosa.

The Salk Institute, La Jolla, CA 92037.


A soluble exogenous antigen reaction (EAR) system has been shown to aid in eating and drinking patterns in rats. The antigen (DA) is intravenously administered directly to the periformal hypothalamus (Williams and Schupf, Neurosci. Abstr.127,1977). An immunological mechanism is suggested by the suppression of EAR in rats at the site the day following EAR treatment. The leucotactic anaphylaxis (AT) CILA might have been produced in a complement cascade initiated by the immune complex of DA. The nonleucotactic CILA would not have been produced. However, and both peptides are cytotoxic via specific receptors, causing smooth muscle contraction, vasokonstriction and degranulation of mast cells. The purpose of this study was to determine the persistent receptor specificity and/or releasing activity of AT on the one hand and, on the other, to eliminate the role of mast cells as a participatory factor. The rats were first sensitized by i.v. injection of DA, and after two weeks intraperitoneally injected with DA, for a period of 2 weeks. For this purpose, we tested the nonleucotactic human AT (CILA) supplied by Dr. A. Hugli for its ability to modify pharmacologically induced appetitive behaviors in rats. The method of sensitization was the same as described by French et al, Proc. Soc. Neurosci. 3:291, 1977. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elic it excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking.

Treating with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking. Treatment with CILA 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CIL-stimulated rats. CILA will elicit excessive eating and stimulation with caroxyl cholesterol will elicit excessive drinking.


The general activity pattern is easily monitored by placing it in an inch of water in a standard, 10 gallon aquarium placed on top of a Selinging activity is recorded as the number of swimming acts completed during a 5 minute period. Activity is measured as an index of activity, goldfish become less active over time and usually generate approximately 300 activity counts during a standard period of 5 minutes. Using this paradigm, goldfish were administered a 5 µl (80 µg/kg) IVC or IP injection of one of 21 test substances and their activity was measured for 30 minutes. Results indicated that activity decreased reliably over time and that significant differences existed as a function of size of injection, with ICV injections producing greater decreases. Of the substances evaluated, no reliable differences existed between the diluent control and melanin, MIP-I, substance P, Met-enkephalin, D-Ala2-enkephalin, D-SIP, and D-Ala3-D-SIP. Significant decreases in activity were obtained using D-Ala2-D-SIP, D-Ala3-D-SIP, and D-Ala2-D-SIP. Further, all endorphins tested produced reliable decreases: d-endorphin, b-endorphin, y-endorphin, D-Leu7-D-Lys19-b-endorphin, D-Leu7-D-Lys19-y-endorphin, and D-Ala2-b-endorphin. Finally, the greatest decreases in activity were produced by two new enkephalin analogs. Further, 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 cross the blood-brain barrier. Accordingly, since both ICV and IP injections produced reliable decreases in activity, it would seem reasonable to test the role of the rate of appearance of the site of injection. Some peptides or their metabolites appeared to readily cross the blood-brain barrier (e.g., Met-enkephalin) as not all were extracted 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

Spinal neurons derived from mouse embryos were grown in tissue culture for 1-2 weeks. Neurons coupled with extracellular iontophoresis were used to study the effects of leucine-enkephalin (ENK) and the putative inhibitory amino acid transmitter 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 membrane in threshold was accompanied by a dose-dependent increase in the current required to attain threshold (threole). This effect was observed in the absence of any change in the current-voltage relations of the cell and clearly illustrated 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. The 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 spikes at these previous phases of the cell were 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 potential away from threshold and by shunting 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 spikes at these previous phases of the cell were increased in a dose-dependent reversible manner. Both amino acid actions desensitized, GABA typically more than glycine.

The effect of leucine-enkephalin (ENK) and the putative inhibitory amino acid transmitter 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 membrane in threshold was accompanied by a dose-dependent increase in the current required to attain threshold (threole). This effect was observed in the absence of any change in the current-voltage relations of the cell and clearly illustrated 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. The 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 spikes at these previous phases of the cell were 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 potential away from threshold and by shunting 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 spikes at these previous phases of the cell were 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 potential away from threshold and by shunting 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 spikes at these previous phases of the cell were 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 potential away from threshold and by shunting 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 spikes at these previous phases of the cell were increased in a dose-dependent reversible manner. Both amino acid actions desensitized, GABA typically more than glycine.

We have recently reported that various neuropeptides distributed throughout the brain and the gastrointestinal tract could act as hypothalamo-neurohypophyseal releasing agents by peripheral injections of capsaicin solutions induced by cold+restraint stress (Taché, Y. and Collu, R., 60th Ann. Meet. End. Soc., 1978). In the present study, we further investigated the relationship 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 (5-0.1 µg) and neurotensin (5µg) further enhanced the hyperglycemic response to stress whereas somatostatin and thyrotropin releasing hormone (TRH) decreased plasma glucose levels in stressed rats; beta-endorphin (5-lµg) 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). Beta-Endorphin (5-lµg) 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 10² 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 to be a very active neuropeptide capable of interfering centrally with stress manifestations.


Constant rate infusion with [3H]tyrosine or [3H]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 in vivo synthesis of MET in striatum may involve the formation of one or more precursors. Since we failed to detect 8-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 8-endorphin. By this antibody, two endorphin-like peptides with molecular weight larger than MET were detected in the eluate 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 endorphins like peptides with antibody directed toward 8-endorphin and 8-lipotropin revealed that the novel endorphins are neither 8-endorphin nor 8-lipotropin. Moreover we have found that after trypsin hydrolysis they can generate a peptide similar to that generated by trypsin hydrolysis of 8-endorphin. With Sephadex G-75 column and thin layer chromatography techniques, we have excluded that the novel peptides are o-endorphins. 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 o- and 8-endorphins and 8-lipotropin. Whether these two peptides function as precursors of MET in striatum will be discussed.
NEUROPHARMACOLOGY
Methysergide (5 mg/kg) shows mixed properties. The changes in the sensitivity to serotonin is thus maximum 3 weeks after spinal transsection. The intensity of the response to 5-HTP can be interpreted in terms of the parallel increase in the activity of the autonomic nervous system and the spontaneous EMG activity of tigh flexors 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 spinal activity induced by 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, ejaculation). 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.

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 herein is 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 brains of 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 O-methyl-bufotenin (OMB) as heptafluorobutyryl derivatives - HFB) exhibits m/e 58.2 as the base peak due to alpha-cleavage of the tertiary amino group. Thus, selection 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 160/200 mesh. Under these conditions the mass fragment ions of DMT and O-methyl-bufotenin are separated in 4 minutes followed by a programmed temperature increase of 10°C/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 Newlett-Packard 5895 A GC/MS data system. Identification of DMT and OMB was based upon retention time and simultaneous monitoring of mass fragment ions 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. Quantity was achieved by an isotope dilution method using alpha, alpha, beta, beta-tetradecuterated 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 #NS01 MW96995-01.

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 in several neuronal pathways including the cortico-striatal projection. With [3H]-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.

The brain membranes were prepared by homogenization of frozen brain in Tris-citrate buffer (0.05 M, pH 7.4 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 Tris-citrate buffer containing 3 μM [3H]-L-Glu in the absence or presence of 100 μM L-Glu; membranes were then isolated by centrifugation. Specific binding is defined as the difference between total binding and non-specific binding in the presence of 100 μM L-Glu. The binding was rapid and reversibly with equilibrium reached by 10 min incubation. The amount of [3H]-L-Glu binding was proportional to membrane protein up to 20 μg. A saturation isotherm for forebrain membranes revealed an apparent Kd of 11 ± 1 nM and a Bmax of 110 ± 15 fmol/mg protein; however, monomeric kinetics occurred at concentrations of [3H]-L-Glu in excess of 10-6 M. The specific binding of [3H]-L-Glu varied in brain regions (fmol/mg protein): parietal cortex, 43 ± 2; frontal, 38 ± 2; hippocampus, 28 ± 2; cerebellum, 13 ± 2; pons-medulla, 10 ± 2. The IC50 for several excitatory amino acids and analogues were examined: L-Glu, 0.7 M; L-aspartic acid, 1.0 M; L-homocysteic acid, 1.0 M; L-glutamine, 1.0 M; L-lysine, 0.2 M; GABA, glycine, taurine, phenytoin, phenobarbital, carbachol, norepinephrine and dopamine were also ineffective at 10 μM.

Deactivation produced a 45 ± 5% reduction in [3H]-L-Glu synaptosomal uptake and a 55 ± 4% reduction in endogenous Glu in the striatum but did not significantly alter the specific binding of [3H]-L-Glu; thus, it is unlikely that [3H]-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 [3H]-L-Glu. These studies suggest that [3H]-L-Glu is binding to a high affinity site primarily localized on neurons that may be a receptor mediating the neurotransmitter action of Glu.

(Supported by USPHS Grants NS 11558, NS 02-00112).

1325 INCREMENTAL DOSES OF MORPHINE PROVIDE METHOD TO IDENTIFY DIFFERENT PATTERNS OF RESPONSES RECORDED FROM EIGHT BRAIN NUCLEI. M. Brown*, B.M. Riger*, and N. Dalby (SPON: T.F. Burks). The University of Texas Medical School at 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), parafascicular thalamus (PF), caudate nucleus (CN), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), lateral septum (Sp), 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 (60um 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, 5, 10, 50 and 100 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).


We have previously reported (Nature 270: 167, 1977) that intraperitoneal injection of 4.0 μmol of morphine effectively antagonized drinking elicited by 3 μmol of d-choline (a non-specific cholinergic agonist). Under these conditions, the effect of morphine was reversed by pretreatment with an equimolar dose of naloxone. In the present experiment, we have extended these data in the presence of 100 μM L-Glu. The binding was rapid and reversibly with equilibrium reached by 10 min incubation. The amount of [3H]-L-Glu binding was proportional to membrane protein up to 20 μg. A saturation isotherm for forebrain membranes revealed an apparent Kd of 11 ± 1 nM and a Bmax of 110 ± 15 fmol/mg protein; however, monomeric kinetics occurred at concentrations of [3H]-L-Glu in excess of 10-6 M. The specific binding of [3H]-L-Glu varied in brain regions (fmol/mg protein): parietal cortex, 43 ± 2; frontal, 38 ± 2; hippocampus, 28 ± 2; cerebellum, 13 ± 2; pons-medulla, 10 ± 2. The IC50 for several excitatory amino acids and analogues were examined: L-Glu, 0.7 M; L-aspartic acid, 1.0 M; L-homocysteic acid, 1.0 M; L-glutamine, 1.0 M; L-lysine, 0.2 M; GABA, glycine, taurine, phenytoin, phenobarbital, carbachol, norepinephrine and dopamine were also ineffective at 10 μM.

Deactivation produced a 45 ± 5% reduction in [3H]-L-Glu synaptosomal uptake and a 55 ± 4% reduction in endogenous Glu in the striatum but did not significantly alter the specific binding of [3H]-L-Glu; thus, it is unlikely that [3H]-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 [3H]-L-Glu. These studies suggest that [3H]-L-Glu is binding to a high affinity site primarily localized on neurons that may be a receptor mediating the neurotransmitter action of Glu.

(Supported by USPHS Grants NS 11558, NS 02-00112).

1327 EXCITATORY EFFECTS OF COCAINE ON THE LIMBIC SYSTEM. Jeremiah P. Collins, Henry Leese 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 hippocampal 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 threshold to hippocampal 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 00803).
1329 EFFECTS OF BARBITURATES ON POSTSYNAPTIC INHIBITORY RESPONSES IN APLYSIA. Ila L. Cote* and W. A. Wilson (SPON: J. Parmentier).

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.

1330 EFFECTS OF BARBITURATES ON POSTSYNAPTIC INHIBITORY RESPONSES IN APLYSIA. Ila L. Cote* and W. A. Wilson (SPON: J. Parmentier).

The effects of barbiturates on responses produced by lontophoretic 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 µM 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.


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, S.D. rats (300-350 g). Different groups of 8-12 animals each received a s.c. injection of 10, 25, or 50 mg/kg, or 0.5, 1, 1.5, 3, or 6 mg/kg, of M or NM, or a s.c. injection of 0.25, 1, 1.5, or 3 mg/kg of N 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. The 0.25, 0.5, 1, or 2 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 then treated immediately after convulsing and plasma samples were obtained, frozen, and subsequently analyzed by GLC for levels of M and NM.

<table>
<thead>
<tr>
<th>Treatment (at 0 hr)</th>
<th>S.T. (at 0.5 hr)</th>
<th>Mean ± S.E. (sec)</th>
<th>Plasma: mean ± s.e. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-S</td>
<td>387 ± 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-M (12.5 mg/kg)</td>
<td>380 ± 12</td>
<td>65 ± 10</td>
<td></td>
</tr>
<tr>
<td>S-N (12.5 mg/kg)</td>
<td>9 ± 12</td>
<td>67 ± 14</td>
<td></td>
</tr>
<tr>
<td>M-M (25.0 mg/kg)</td>
<td>636 ± 10</td>
<td>124 ± 30</td>
<td></td>
</tr>
<tr>
<td>M-N (326 ± 10</td>
<td>284 ± 10</td>
<td>123 ± 23</td>
<td>100 ± 18</td>
</tr>
<tr>
<td>M-NM (12.5 mg/kg)</td>
<td>1025 ± 10</td>
<td>1253 ± 79</td>
<td></td>
</tr>
<tr>
<td>S-M (25.0 mg/kg)</td>
<td>309 ± 9</td>
<td>2687 ± 365</td>
<td></td>
</tr>
<tr>
<td>S-NM (25.0 mg/kg)</td>
<td>284 ± 10</td>
<td>2767 ± 345</td>
<td></td>
</tr>
</tbody>
</table>

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 M or NM.

(Supported by grants DA 00376, 01707, and 01457 from NIDA.)
1332 ELECTROPHysiOLOGICAl EVIdENCE FOR TOLERANCE TO MORPHINE: unIt ACTIVITY RECORDInG FrOM HypOTHALAMUS AND PARAFAncricUS NUCLEI IN FREELY MovInG rATs. N. DaPpy and B.M. RieOR**. 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 multunit activity in naive rats. The present experiment was intended to examine 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 (8) of the VMH units. Permanent electrodes (60 μm in diameter) were implanted previously in the parafascicularus (PF) and within the ventromedial hypothalamus (VMH) under a stereotaxic protocol. Naloxone (1 mg/kg) was injected on day 1 (naive animals) and spontaneous multiunit activity in naive rats. The present experiment was responded on day 1 with increased activity, exhibited at day 5 a variety of 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 neuropharmacological evidence of tolerance to morphine, but the pattern of tolerance in each structure was different. (Supported by USPHS DA 00803)

1333 ANATOMical AND ELECTROPHYSiOLOGICAl EVIdENCE OF NEuRONAL ACTIVITY IN NEURONS OF the NUCLEUS A10 REGION OF the RAT. M. Delsen*, D.C. German and R.S. Kiser (SPON: H. Hoffwarp). Dept. 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 rat, the anatomical (morphological) staining was used to identify A10 neurons (6-105 CI/mole) was iontophoretically ejected into the A10 region, and the ascending projections of these cells were subsequently traced autoradiographically. Neurons were considered to have long action potential durations (> 2 msec.), and histologically localized within the A10 region that 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 (Runney et al., 1973). These cells were antidromically activated, by stimulation in the nucleus accumens area, as judged by either constant latency, or collateral 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 compacta (Guyenet & Aghajanian, 1977). These slowly-conducting fibers are often said to be afferent to the nigra or subthalamic region (A10).

Other cells in the A10 region were orthodromically activated at longer latencies than cells which were antidromically activated (latency > 25-30 msec). 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) these cells may also be orthodromically activated by descending fibers to nucleus A10. (Supported by USPHS grant MH-27574.)


Monoamine 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 to be 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 neuronal accumulation.

Accumulation of [3H]-DA was examined in rat brain synaptosomes. MAO activity was determined in sucrose lysed rat brain synaptosomes. It was found that highly specific MAO blockers could not be distinguished from each other on the basis of their action on MAO activity. 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 rat, the anatomical (morphological) staining was used to identify A10 neurons (6-105 CI/mole) was iontophoretically ejected into the A10 region, and the ascending projections of these cells were subsequently traced autoradiographically. Neurons were considered to have long action potential durations (> 2 msec.), and histologically localized within the A10 region that 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 (Runney et al., 1973). These cells were antidromically activated, by stimulation in the nucleus accumens area, as judged by either constant latency, or collateral 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 compacta (Guyenet & Aghajanian, 1977). These slowly-conducting fibers are often said to be afferent to the nigra or subthalamic region (A10).

Other cells in the A10 region were orthodromically activated at longer latencies than cells which were antidromically activated (latency > 25-30 msec). 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) these cells may also be orthodromically activated by descending fibers to nucleus A10. (Supported by USPHS grant MH-27574.)


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 the DA antipsychotics (e.g., low-dose chlorpromazine) increase DA levels in synaptosomes from rat brains, and that the DA autoreceptor density in the substantia nigra is reduced by chronic treatment with the DA antagonist, pargyline. These findings, along with the recent reports suggesting that morphine produces a depression of spontaneous activity in the rat, indicate that morphine may act as an antagonist at the DA autoreceptor site. In addition, the effects of morphine on DA transmission in the rat may be mediated by an interaction with the DA receptor sites in the basal ganglia. The present study was designed to test the hypothesis that morphine may act as an antagonist at the DA receptor sites in the basal ganglia. The results of this study are presented in three sections: (1) identification of the DA receptor sites in the basal ganglia; (2) the effects of morphine on DA transmission in the rat; and (3) the effects of morphine on DA transmission in the rat. 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 rat, the anatomical (morphological) staining was used to identify A10 neurons (6-105 CI/mole) was iontophoretically ejected into the A10 region, and the ascending projections of these cells were subsequently traced autoradiographically. Neurons were considered to have long action potential durations (> 2 msec.), and histologically localized within the A10 region that 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 (Runney et al., 1973). These cells were antidromically activated, by stimulation in the nucleus accumens area, as judged by either constant latency, or collateral 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 compacta (Guyenet & Aghajanian, 1977). These slowly-conducting fibers are often said to be afferent to the nigra or subthalamic region (A10).

Other cells in the A10 region were orthodromically activated at longer latencies than cells which were antidromically activated (latency > 25-30 msec). 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) these cells may also be orthodromically activated by descending fibers to nucleus A10. (Supported by USPHS grant MH-27574.)
1338 1339

NALOXONE AND THE EFFECTS OF SYSTEMICALLY ADMINISTERED KAINIC ACID. 1337 1338
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 stratum moleculare of either
anesthetized with 75% nitrous oxide. Cells were driven by
physiological stimuli and their receptive field characteristics

The evoked activity of most cells was enhanced or depressed by LSD. Enhancement occurred with small injections (0-10 nA); while depression was observed at 0-10 nA, but more frequently
when currents >10 nA were used. Also inconsistent changes in
unstimulated background firing were observed. In some neurons there was a clear pattern of disinhibition. Tolerance of LSD appeared in many cells after repeated administra-
Tolerance of LSD appeared in many cells after repeated administra-
tions. Methysergide sometimes produced similar effects to LSD, but the response to this drug was weaker and required more prolonged administration with higher currents (30-60 nA). 2-Bromo-LSD (BOL) however, was generally inactive on cells which LSD had clearly inverted. 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 quite sensitive to LSD. Since the effects of LSD were miniscule and 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; NIH NS-09156, DA-01458; and NIH NS-09156, DA-01458; and NIH NS-09156, DA-01458; and NIH NS-09156, DA-01458; and NIH NS-09156.

1338 1339

1338 - 1339

JOINTPHORESIS OF LSD: EFFECTS ON RESPONSES OF SINGLE CORPECTAL NEURONS TO VISUAL STIMULATION. P. C. Fox and A. Drury.

1338 - 1339

1338 - 1339


The present studies compare the specificity of several putative
ethanol antagonists for benzodiazepines (BZ)-mediated anticonvulsant (BZ) actions, fenmetozole (F), and apomorphine (A) against a spectrum of ethanol actions. These included ethanol-induced impairment of olive body rhythmicity, increased locomotor activity, and increased shock-punished drinking in water-deprived rats, decreased cerebel-
lar granule 3',5'-monophosphate (cGMP) and physical depen-
dence.

Ethanol (3 g/kg, ip) impaired motor coordination in an air
borne righting reflex test. Treatment with TRH (20-80 mg/kg) or F (15-30 mg/kg) significantly reversed ethanol induced impair-
ment, whereas A (3-10 mg/kg) was without effect. A proposed "stimulatory" action of ethanol (2.0 g/kg, ip) to increase spontane-
ous locomotor activity was observed in Wistar/Jcr 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 physical dependence.

The observed differences in interactions of purported "ethanol antagonists" compared across a spectrum of ethanol's actions indicate that BZ 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.

1339 - 1338

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% O₂ and 5% CO₂. Naloxone (10⁻⁶ 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 2x10⁻⁶M atropine, a concentration sufficient to abolish contractile response to 10⁻⁴M acetylcholine. To examine the possibility that 5-HT was involved in mediating the atropine resistant contraction the response to naloxone (10⁻⁶ M) was examined after 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.

EFFECTS OF ETHANOL ON THE SPONTANEOUS ACTIVITY OF SINGLE UNITS IN THE HIPPOCAMPUS OF THE AMAZE SEMI-RESTRAINED RAT. Larry A. Grupp. Department of Pharmacology, University of Toronto, Toronto, Canada, MSS IA8 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 interdose 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 "beta" 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.


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, the 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 mottility were observed.

Forty male Sprague-Dawley rats received chronic bilateral implants of 25-gauge 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-gauge injection cannula into the substantia nigra. Movement frequencies were observed for 60 min after 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.
1346 EFFECTS OF ETHANOL, MORPHINE, AND PENTOBARBITAL ON CALCIUM LOCALIZATION IN BRAIN. W. E. Hood* and R. Adron Harris,
Depart. 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. 20:301, 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 water and 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 synaptic calcium content (p<.001) and chronic pentobarbital (75 mg/kg i.p.) decreased mouse brain synaptic calcium by 17% (p<.02). Moreover, acute pentobarbital decreased the calcium content of myelin 27% (p<.01) and the extra-synaptosomal mitochondria 17% (p<.001). However, the acute administration of ethanol to rats (5 g/kg i.p.) or to mice (10 g/kg) failed to change the calcium content of 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^-4 M; End's Dewey, Fed. Proc. 37:763, 1978) and pentobarbital (1.5% M; Blaustein & Ector, Mol. Pharmacol. 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 65 % (p<.02). In addition, ethanol withdrawal produced a decrease in rat myelin magnesium of 10% (p<.05). Also, mice given ethanol withdrawal showed a decrease in serum magnesium and an increase in free fatty acids in agreement with clinical studies of alcoholic cirrhosis. The changes from ethanol and mice chronically exposed to pentobarbital showed no change in calcium levels in any of the brain fractions.

The results from the in vitro and in vivo studies suggest that the lower calcium content of synaptosomes following acute pentobarbital and morphine may be related to the inhibition of the dopamine receptor. It was also implied that a decrease in synaptic 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 EFFECTS OF ETHANOL, MORPHINE, AND PENTOBARBITAL ON CALCIUM LOCALIZATION IN BRAIN. W. E. Hood* and R. Adron Harris,
Depart. 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. 20:301, 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 water and 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 synaptic calcium content (p<.001) and chronic pentobarbital (75 mg/kg i.p.) decreased mouse brain synaptic calcium by 17% (p<.02). Moreover, acute pentobarbital decreased the calcium content of myelin 27% (p<.01) and the extra-synaptosomal mitochondria 17% (p<.001). However, the acute administration of ethanol to rats (5 g/kg i.p.) or to mice (10 g/kg) failed to change the calcium content of 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^-4 M; End's Dewey, Fed. Proc. 37:763, 1978) and pentobarbital (1.5% M; Blaustein & Ector, Mol. Pharmacol. 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 65 % (p<.02). In addition, ethanol withdrawal produced a decrease in rat myelin magnesium of 10% (p<.05). Also, mice given ethanol withdrawal showed a decrease in serum magnesium and an increase in free fatty acids in agreement with clinical studies of alcoholic cirrhosis. The changes from ethanol and mice chronically exposed to pentobarbital showed no change in calcium levels in any of the brain fractions.

The results from the in vitro and in vivo studies suggest that the lower calcium content of synaptosomes following acute pentobarbital and morphine may be related to the inhibition of the dopamine receptor. It was also implied that a decrease in synaptic 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.
1348  SEROTONERGIC-DOPAMINERGIC EFFECTS OF ERGOT DRUGS, S. Kennedy*, R. Hruska & E. Silbergeld, NINCDS, NIH, Bethesda, MD 20014.
In the basis of behavioral assays, such as rotation, and in the absence of parietal nigral lesions, several new ergot derivatives are proposed to possess dopaminergic properties. However, the indirect dopamine systems suggest dopaminergic activity in the basal nuclei. We have studied the effects of the following ergot drugs: bromocriptine, lergotrile, lisuride, metergoline, and Sandoz 25-397, 29-712, and 29-717. These drugs were studied in rats which had been pretreated with a high dose of amphetamine (AMP) and were used to express stimulation of dopamine (DA) pathways; the "serotonic syndrome" (Jacobs, Life Sci. 19:777, 1976), which may reflect stimulation of serotonin (5-HT) pathways, or decreasing rates at a wide range of stimulating currents (10-120 µA). AFA (0.05, 0.1, and 0.2 mg/kg) was compared to the effects of AMP (0.25, 0.5, and 0.5 mg/kg) in increasing barpressing 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 compared to AMP (0.25, 0.5, and 0.5 mg/kg) in increasing barpressing rates. In the third study, animals were pretreated with the DA synthesis inhibitor, a-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats. In the fourth study, animals were pretreated with the DA synthesis inhibitor, a-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats. In the fifth study, animals were pretreated with the DA synthesis inhibitor, a-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats.

The results suggest that the ergots possess significant but varied behavioral effects in systems known to involve DA and 5-HT modulation. The separation of DA from 5-HT in producing these effects is difficult, and this complicates the definition of the ergot drugs as relatively dopaminergic or serotonergic. However, the effects of 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 "seroptonic syndrome" and may thus possess mixed actions on DA and 5-HT pathways.

Drugs that antagonize inhibitory effects of GABA in the CNS (picrotoxin, bicuculline, and muscimol) are used to study the role of GABAergic mechanisms in the functioning of the CNS. We have studied the effects of picrotoxin and bicuculline on the spontaneous occurrence of wave-spike EEG activity. In one experiment, wave-spike discharge was triggered by light-stimuli and recorded from occipital cortex in freely moving rats. The injection of GABA-antagonists caused a decrease in the frequency of wave-spike EEG activity. In a second experiment, picrotoxin, bicuculline, and muscimol were administered to rats. These results will be discussed in terms of a correlation between the ability of drugs to produce wave-spike EEG activity and the depressant effect of the drug on the ongoing EEG and behavior. The effects of wave-spike EEG activity are being studied in terms of hypothesises to explain the paradoxical effect of the GABA-agonists.

Amphetamine (AMP) both potentiates the function of the dopamine (DA) neuron, but they do so by different means. Whereas AMP increases the release of newly-synthesized DA, AFA's action is not dependent upon the availability of newly-synthesized DA. Rats were stereotactically implanted with chronic bilateral 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 barpressing 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 compared to AMP (0.25, 0.5, and 0.5 mg/kg) in increasing barpressing rates. In the third study, animals were pretreated with the DA synthesis inhibitor, α-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats. In the fourth study, animals were pretreated with the DA synthesis inhibitor, α-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats. In the fifth study, animals were pretreated with the DA synthesis inhibitor, α-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats. In the sixth study, animals were pretreated with the DA synthesis inhibitor, α-methyl-α-phenyl-2-pyridylethylamine (α-MP), and AMP was compared to AMP-pretreated rats.

The results suggest that the ergots possess significant but varied behavioral effects in systems known 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, 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 "seroptonic syndrome" and may thus possess mixed actions on DA and 5-HT pathways.

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, 104, 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 hypertensive rats (Zavatski, F. G. & Wurtman, R. J., J. Pharm. Pharmacol., 30, 66 (1978)).

Such results indicate that a similar alteration of the central actions of aldomet may occur in response to hyperamino acidemia 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 triptophan (which decreased by 50%) increased markedly in diabetic animals compared to controls. Branched-chain NAA (i.e., leucine, isoleucine and valine) were increased, whereas alanine was unchanged. 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 showed improvement of aldomet 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 triptophan (which decreased by 50%) increased markedly in diabetic animals compared to controls. Branched-chain NAA (i.e., leucine, isoleucine and valine) were increased, whereas alanine was unchanged. 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 showed improvement of aldomet brain entry and hypotensive action of aldomet 3 days later.
It was demonstrated previously that tryptophan hydroxylase (TOH) from corpus striatum, an area rich in serotonergic cell bodies, is activated in vitro by calcium (Ca++) at concentrations of 1.0 mM or greater (Lange and Haddad, 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 putrescine cofactor, putrescine, and for substrate tryptophan. However, concentrations of 10^{-6} M to 10^{-7} M Ca++ in which cofactor and substrate are saturating to determine TOH activity, we now report that BCaTA pretreatment of midbrain TOH followed by dialysis for 16 hours without EGTA pretreatment or allowed to stand at 20°C for 1 to 2 hours. Subsequently we examined TOH from corpus striatum, an area rich in serotonergic nerve endings, to determine its response and sensitivity to Ca++. In response to intermediate Ca++ concentrations (~200 μM) this preparation (without ECTA pretreatment, dialysis, or incubation at 20°C) demonstrated increased activity resulting from augmented Vmax and no change in affinity for cofactor or substrate.

TOH activity has been reported to be increased by elevated Vmax following an acute load of phenylalanine (Stackebrandt et al. Pharmacol. Exp. Ther. 201: 110, 1977), and a "prolonged" activation of tyrosine hydroxylase (TOH) also by increased Vmax has been reported following significant stimulation of the hypothalamus (e.g., from dietary changes) (Nature 258: 440, 1975). In neither case could changes in the enzyme's affinity for cofactor or substrate be shown. Immunochemical stimulation of the hypothalamus in TOH activation by rather than an increase in enzyme molecules. The TOH activation by physiological concentrations of Ca++ which we have demonstrated, i.e., an effect resulting from increased Vmax 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.


The interaction of potential agonists with the postsynaptic γ-aminobutyric acid (GABA) receptor was investigated physiologically and quantitatively in a lesioned isolated crusiate neuron. The crayfish slowly-adapting, abdominal stretch receptor neuron, which receives direct GABAergic inhibitory innervation, was tested with constant current depolarizing pulse and impaled with two intracellular electrodes for monitoring membrane input conductance. Increase in conductance, the postsynaptic activity of GABA was measured for bath-applied GABA and compared with the effect of various structurally-related compounds. Non-cumulative ion concentration vs. conductance change (μS) plots were obtained, and in each case the contribution 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>(−)-α-methyl-γ-aminobutyric acid=3-amino-propane sulfonate>(+)-α-methyl-γ-aminobutyric acid=isonipeptid acid. All agonists acted in a dose-dependent manner with no apparent order. The Marine Biomedical Institute, and Dept. of Human Biological Chemistry & Genetics and Dept. of Neurology, The University of Texas Medical Branch, Galveston, Texas, 77550.

1353 EFFECTS OF GABA AND STRUCTURALLY-RELATED COMPOUNDS ON CONDUCTANCE OF CRAYFISH ABDOMINAL STRETCH RECEPTOR. Glenda H. Kraege, Katu Inaba and Eugene Roberts, Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010.

Electrical and metabolic responses to direct cortical stimulation were measured in cats administered ethanol and acetaldehyde i.v. Stimuli were presented in pulsed trains of 1-2 sec at a shift of steady potential up to 6mV and a decrease in the ratio of reduction/oxidation of the mitochondrial respiratory components. The latter were measured noninvasively by reflection microfluorometry and spectrophotometry. The rates of oxidation and the ratio of reduction of these components are slowed by ethanol and acetaldehyde. The slowing of oxidation is similar to the effect of ouabain when microinjected unilaterally into the brain. It selects 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 slow 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-reduction ratio of the mitochondrial electron transport chain. It appears that the former was dependent on the latter being independent of the integrity of certain forebrain structures. The present investigation provides further evidence for a location within the brain stem. Thus, APO activation of AS was eliminated, but inhibition was unaffected in animals subjected to preanamnial complete or partial transection or electrocoagulation (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 (0.5 mg/kg, i.v.) also blocked the inhibition of AS by modulate metabolites acetate and propionate. The inhibition of AS by modulate metabolites acetate and propionate was shown to be surmountable. However, recovery was not evident even after periods up to 9 hours.


Previous experiments have shown that automatic swallowing (AS) in the unrestrained anesthetized rat is influenced by the injection of dopaminergic and serotoninergic activity in the brain (Bieger et al., 1977, Neuropharmac., 16:245). The dopaminergic apomorphine (APO) exerts both an excitatory and as well as a depressant effect on AS. The former being dependent, the latter being independent of the integrity of certain brainstem structures. The present investigation provides further evidence for a location within the brain stem. Thus, APO activation of AS was eliminated, but inhibition was unaffected in animals subjected to preanamnial complete or partial transection or electrocoagulation (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 (0.5 mg/kg, i.v.) also 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 for only about five minutes in the absence of dopamine in the brain stem. Thus, APO activation of AS was accompanied by an increase in respiratory frequency. Animals pretreated with massive intracerebroventricular doses of 5,6-dihydoxydopamine (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 play a role in the control of swallowing. These receptors do not appear to be prejunctional with respect to local catecholamine neurons, as postulated by other workers in reference to blood pressure control. These results extend to other dopamine receptors, the idea of local catecholamine neurons, with postganglionic sympathetic neurons mediating their effects, as postulated by other workers in reference to blood pressure control. The possibility of these dopamine receptors in the production of involuntary movements by neuroleptic drugs calls for careful examination.


A class of naturally occurring tetrahydroxyquinolines, norlaudanosolinecarboxylic acids (NLCA's), were found to be competitive inhibitors of dopamine uptake into synaptosomes and cytochrome-c oxidase. The latter were measured noninvasively by reflection microfluorometry and spectrophotometry. The rates of oxidation and the ratio of reduction of these components are slowed by ethanol and acetaldehyde. The slowing of oxidation is similar to the effect of ouabain when microinjected unilaterally into the brain. It selects 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 slow 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-reduction ratio of the mitochondrial electron transport chain. It appears that the former was dependent on the latter being independent of the integrity of certain forebrain structures. The present investigation provides further evidence for a location within the brain stem. Thus, APO activation of AS was eliminated, but inhibition was unaffected in animals subjected to preanamnial complete or partial transection or electrocoagulation (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 (0.5 mg/kg, i.v.) also blocked APO inhibition, this antagonism being surmountable. However, recovery was not evident even after periods up to 9 hours.

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 decreased to 80% at 4 hr. Inhibition of forelimb extension was chosen as an end-point for MCL actions on seizures in vivo. The toxin forelimb extension component of BIC seizures was abolished by MCL (ED50=1.0 µg/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>Picrotoxin>phenobarbital>diphenylhydantoin. MCL had no effect on strychnine-induced convulsions in doses up to 8 mg/kg i.v., however, it was 25 times as potent as picrotoxin in this test. The time course of anticonvulsant activity of MCL (2 mg/kg, i.v.) against BIC seizures was determined. Maximal protection (93% of rats tested) occurred 15-30 min after MCL and gradually declined over 300 min. 14C-MCL and MCL 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 14C concentration (0.18±0.02 µg/g tissue) occurred 30 min after 14C-MCL. Radioactivity remaining over time showed a biphasic decline over 300 min. Twenty-four hours post injection 0.04±0.01 µg/g of radiolabel remain in the brain. Thus peak brain concentration of radioactivity occurs 30 min after intravenous administration of 14C-MCL and is maintained over time 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 metabolites antagonizes BIC blockade of GABA receptors.

We have characterized lithium chloride's (LiCl) effects on choline transport into the brain using the Brain Uptake Index (BUI) technique described by Oldendorf et al. (1976). 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 3H-labeled test substance to the passive diffusion of 3H$_2$O.

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 transport 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% for controls to 5.95 ± 0.36% and 4.56 ± 0.48% for 5mM and 10mM); 3) Chronically administered LiCl, given in the rats' diets, elevates serum lithium levels and decreases choline transport into the brain using the Brain Uptake Index. 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**

<table>
<thead>
<tr>
<th>Dose of LiCl</th>
<th>% Choline Extraction</th>
<th>Serum Lithium</th>
<th>mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.71 ± 0.46*</td>
<td>1.69 ± 0.61</td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>5.94 ± 0.45</td>
<td>2.45 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>4.63 ± 0.53</td>
<td>2.45 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>3.57 ± 0.18</td>
<td>6.71 ± 0.78</td>
<td></td>
</tr>
</tbody>
</table>

*All values expressed as ± 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 5%. 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 reduced the uptake of choline by 50%.

Our observation that acute or chronic lithium administration decreases choline transport into the brain demonstrates the importance of lithium as an agent with central cholinergic neurotransmission.


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 do not induce 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 have proved of limited usefulness in 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 do not induce rigidity (e.g., neuroleptics: Costall and Naylor, 1974).


Lidocaine (L) is a synthetic local anesthetic 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, have been reported which suggest that L has a central stimulant-like activity. L has been shown to be a weak dopaminergic agonist and it has been found to increase amounts of tritium in DNA of morphinized rats, even though there was less tritium in supernatants. There was an increase in DNA levels, but the increase was not greater in the supernatants of 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 3H into striatal DNA cannot be a consequence of greater access of thymine to the striatum. The increase of thyamine incorporation into DNA in the striatum probably reflects an increase in cell proliferation induced by morphine. Supported by grant DA 01685.

ACUTE MORPHINE ADMINISTRATION INCREASES INCORPORATION OF 3H- THYMIDINE INTO BRAIN STRIATAL DNA. Rita B. Weisinger, Jack C. Hewire*, Gary S. Lynch, Sam A. Deshazer and Cort Flitchbaugh, Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717.

The extremely long time-course of some morphine effects suggested that opiate agonists persist in oligodendroglia, a glial cell type found in brain. One possibility is that morphine induces changes in glial cell proliferation. For this reason we undertook a study of the effects of acute morphine administration on thymidine FRACTIONS OF SLOW-RELEASE PREPARATION OF MORPHINE. HCL or vehicle and 1 mg/kg of naloxone HCl or vehicle 30 and 45 min, respectively, prior to administration of 600-900 mg/kg of 3H-thymidine, or vehicle or 1 mg/kg of naloxone. As predicted, the high dose of morphine produced significant sedation (16% at 200 mg/kg and 45% at 300 mg/kg). Morphine produced a time-dependent increase in the rate of DNA synthesis with a peak response at 15 min prior to 3H-thymidine administration. Sera were sacrificed 1 h after thymidine injections and DNA fractions were isolated from tissues. Acute morphine administration did not increase incorporation of 3H-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 3H-thymidine incorporation into DNA was observed in any area of the brain of morphine-addicted rats or in rats undergoing withdrawal. In spleens, however, morphine incorporation was decreased to 60% of control in rats undergoing naloxone-precipitated withdrawal. To see if the observed changes in incorporation of 3H-thymidine into DNA by striatum can be accounted for by differences in the local availability of the label in morphine-injected rats, amounts of tritium in DNA were correlated with amounts in supernatants of the tissue homogenate. Again, the 3H incorporation was higher in striatal DNA of morphineized rats, even though there was less tritium in supernatants. There was an increase in DNA levels, but the increase was not greater in the supernatants of 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 3H into striatal DNA cannot be a consequence of greater access of thyamine to the striatum. The increase of thyamine incorporation into DNA in the striatum probably reflects an increase in cell proliferation induced by morphine. Supported by grant DA 01685.

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 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 [3H]-tyrosine to [3H]-DA or -norepinephrine (NE) for at least 5 hours in the presence of air or CO. One hr after injection the rats were exposed to CO (1700 ppm), hypoxia (7.5% O2) 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 exposure to CO 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 48 hr after injection of 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, NE turnover in saline treated rats exposed to 1000 or 1500 ppm CO were significantly elevated above levels in the saline treated 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 dependent on the receptor. 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).


Antidepressant drugs bind specifically to rat brain synaptoplasmic fractions. The binding, using [3H]-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 well with clinical efficacy but not with the inhibition of monoamine uptake. Thus, the clinically-active antidepressants, iprindole and mianserin, which are active in inhibiting [3H]-imipramine binding (IC50's of 7 and 48 µM, respectively) are inactive in standard antidepressant evaluations such as inhibition of monoamine uptake and prevention of tetrazenes in vivo. Amphetamines and other more recently described anergic 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 [3H]-imipramine bound. In contrast, to other types of specific drug receptor binding (e.g. benzodiazepines) 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 synthesis of new antidepressant-like compounds in brain.


The segmental reflex system for the low spinal cord has long been an important model system for studying drug effects in 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, 1960; Frijns & Hoekstra, Arch. Int. Pharmacol., 1970; Noyer-Lohmann, Arch. Pharmacol., 1972; Lathers & Smith, JPET, 1976). no studies have been reported on ethanol withdrawal. Therefore, the present investigation 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 denervation, including spontaneous grand mal type convulsions 24 hours after abrupt withdrawal of ethanol. The intensity of withdrawal signs, electroeurophysiological measurements of spinal cord activity were made. The method used in the present study has been published elsewhere (2) to compare the results of ethanol withdrawal (Rosenberg & Okamoto, JPET, 1976, 1978). The excitation function measured by the amplitude of 2K spikes, polysynaptic discharge pattern, 2K discharge zone, frequency resonance curve for posttetanic 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).


Rats, under Equithene 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 telencephalon, thalamus, globus pallidus and septum) was removed by suction, resulting in the so-called "thalamic rat" preparation. 24 hours after abrupt withdrawal of the animals (1) to uncover the neuronal alteration produced during 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).
IBOTENIC ACID: EXCITATORY AND INHIBITORY ACTIONS IN BRAIN. E. P. H. Blackwell and E. P. H. Blackwell. NIDA Addiction Research Center, Lexington, Kentucky 40503.

We have found that ethylketocyclazocine (Win 35,197-2), an orally active disassociative agent similar to ketamine, caused an increase in spontaneous neuronal activity. This increase was followed by a decrease in neuronal firing rate. The excitatory effects were mediated by opioid receptors, while the inhibitory effects were due to a decrease in glutamate release. These results suggest that ibotenic acid, a close relative of muscimol, has a dual action on neuronal receptors: a relatively fast excitation with a much slower time course than is seen when glutamate is applied (a long latency of onset and prolonged action), and a powerful and very prolonged depression of glutamate-evoked activity. This was produced by releasing ibotenic acid from a microiontophoretic electrode. The effects of ibotenic acid were antagonized by naloxone, a potent opioid receptor antagonist. These results confirm previous findings in the spinal cord and suggest that ibotenic acid may have therapeutic potential in the treatment of pain.


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 action). After-discharge was more prominent in cats under halothane than under methoxyflurane anesthesia. Even minute, subthreshold amounts of ibotenic acid caused stereotyped head bobbing. Phencyclidine (0.1, 0.25 and 1.0 mg/kg) caused stereotyped 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 / agonists and that phencyclidine has opposite effects which are more amphetamine-like.


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, Stanley and Wilk, Eur. J. Pharmacol., 1977, 44: 293). We have found that administration of haloperidol and clozapine to the rat produces a dose-dependent increase in firing rate in the neostriatum which ranged in magnitude for individual neurons from 200-800 percent. Comparative results were obtained in the accumbens nucleus. In a separate group of animals, i.p. administration of 25 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 homogeneous, have important implications for the behavioral pharmacology of the neuroleptics and related drugs. Further studies related to the actions of haloperidol and clozapine, may have important implications for the treatment of psychiatric disorders and related conditions.

This research was supported, in part, by Biomedical Research Support Grant #56124-02 from the Indiana University.
1372 MICROIONTOPHORESIS OF INHIBITORY AMINO ACIDS IN THE MEDIAL HYPOTHALAMUS: EVIDENCE FOR GABA AS AN INHIBITORY HYPOTHALAMIC NEUROTRANSMITTER. Leo F. Remage, Quentin J. Flitton and Howard V. Blume. Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada, H3C 1A6.

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 in a recurrent or divergent pathway. 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 transphenyeal 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. -glutamate and -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: -glutaminoenopropionic acid (G-6P) > GABA > -alnine > -aminovaleric acid (AVA) > glycine. Simultaneous microiontophoresis of an agonist and antagonist to postsynaptic inhibition evoked by these agents elicit an initial excitatory effect, with a possible antagonistic effect on postsynaptic inhibition evoked by these agonists. This latter action usually required administration of an agonist at a dose that was less than the minimal effective dose. The report concludes with a discussion of the possible pharmacology of these pathways. Acknowledgements: Supported by Medical Research Council of Canada.


Cadaverine (1,5-diamino-pentane) and its acylated derivatives occur in mammalian brain (Belezaelova et al., 1974; 1977) but a physiological function has not been assigned to these compounds. Cadaverine has been shown to be taken up by brain slices (Piccoli, 1972) and along with its acyl derivatives (belezaelova et al., 1974) it is present in the serum and blood of schizophrenics (Ferry, 1967; Belezaelova, 1977). Single cell microiontophoresis studies by our group showed that while cadaverine had no effect, the membrane depolarization of its acyl derivatives caused a long-lasting, slowly decreasing depolarization (Miller, 1977). It was suggested that masking of one of the enzymes involved in the metabolism of cadaverine by acylating agents would alter the excitability of these neurons. The present study details our attempts to test if the excitability of neurons is affected by the acylated derivatives of cadaverine. Both observations suggest that hypothalamic neurons have both GABA and glycine receptors, but that GABA is the more like­ly to excite these cells. (Supported by the MRC).

1375 DIFFERENTIAL EFFECTS OF ANESTHETIC-LIKE DRUGS ON ISOLATED NEURON PREPARATION. Sheldon R. Roth and Bruce M. Maciver*. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1A4.

The isolated abdominal muscle receptor organ (MBD) 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 common 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 MBD 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, pentoabarbitol, methohylate and halothane. All the agents are capable of depressing the characteristic output activity of the MBD 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 discharges.

Harmaline, at concentrations of the order of 10-6 M changes the characteristic single action potential output potential to a rhythmic doublet or paired activity. Ethanol (100 mg/kg, i.p., 2 hr. prior) produces similar effects at concentrations as low as 0.12 M, and at 10 M produces a periodic discharge of high frequency bursts, consisting of between 7 to 15 spikes/ burst (Kiser, 1977). Other hallucinogenic drugs, such as mescaline, have been shown to be taken up by brain slices and to be present in the serum and blood of schizophrenics (Perry, 1967, Dolezalova, 1977). The studies were supported by Medical Research Council of Canada.


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 when NE neurons are stimulated (Kiser et al., 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-pa­ramine (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., Geman 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 electrophysiologgical characteristics as re­ported by Cadet & German (1977). Input stimulus (i.e. stretch) is varied and quantitatively measured via a sensitive strain gauge while simultaneously recording neuronal activity. 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 Cadet & German (1977).
1376 ALTERATIONS OF CENTRAL AUTONOMIC CARDIOVASCULAR RESPONSES BY Δ9-THETAHROIDOCANNABINOL. William T. Schmeling* and Michael J. Mosko. Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

Δ9-Tetrahydrocannabinol, the primary psychoactive component of marijuana, has been shown 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 Δ9-THC to induce hypotension is not compromised by transection of the neurethral at the midecullular level but is abolished by high cervical section. Experiments were conducted in rats and cats to determine the effect of Δ9-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 level. 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 conducted in rats and cats to determine the effect of Δ9-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 level. 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 conducted in rats and cats to determine the effect of Δ9-THC. These findings are consistent with the hypothesis that Δ9-THC elicits significant disruption of sympathetic and parasympathetic tonic homeostatic cardiovascular control mechanisms. (Supported by USPHS NIH Grant DA00124.)


Parenteral administration of choline (Ch) to rats has been shown to alter the effects of other drugs and in some cases to induce biochemical changes in the brain. The authors have therefore examined the effects of chronic dietary and parenteral Ch administration on behavior. Male Sprague-Dawley rats (215 g) were maintained for 4 weeks on a semipurified diet, 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, Ch (60 mg/kg free base), atropine sulfate (20 mg/kg) or Ch followed by atropine at 60 min. Rats were placed in a symmetrical 1-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<0.05) 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 blocked; whereas the atropine 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 (Ch injected). 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 mediation of activity in rats in response to Ch. In addition, we have extended our previous observation on the modification of the central actions of atropine by Ch pretreatment to include behavioral and biochemical parameters, the nutritional status of an animal, i.e., Ch availability, may be significant in determining the efficacy, both behavioral and biochemical, of pharmacological agents such as Ch in the central nervous system. Further studies will hopefully elucidate the specific mechanisms involved in this interaction. (Supported by a NIMH Research Support Grant RR-05424-16 and an NIMH Grant MH-29182.)

1378 A TONIC MUSCARINIC INHIBITORY INPUT TO PUPILLOCONSTRICTOR NEURONS IN THE EDINGER-VESTRAL NUCLEUS OF THE DOG. Lawrence G. Sharpe and Wallace R. 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, during which time no signs of conscious discomfort (30 ga inner, 23 ga outer, insulated except at the tips) was observed. The cannula guide into a site where electrical stimulation (20 Hz, 0.5 msec, 2-4 V) had evoked maximal pupil response with ocular movements. Drugs dissolved in sterile saline were injected in a 0.5 ml volume over 1 min into this site. The injection cannula 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 mmol), in 1.0 µl completely antagonized the mydriatic activity of carbachol (0.1 µg) and physostigmine (5 µg). Microinjections of the nicotinic antagonist mecamylamine (2.73 mmol) and hexamethonium (2.73 mmol) 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 miosis. The mydriatic activity of the cholinergic inhibitory pathway was abolished by anticholinergic agents.

These data suggest that the tonic inhibitory input to the EW nucleus is muscarinic. Drugs that act centrally to produce miosis (morphine, hexamethonium) 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, Biocal 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 (0.03 mg/kg, s.c.) on the injection-induced DA cholinergic response in DA. First, we examined the effect of this 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 60% while chronic 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 investigated the ability of fluphenazine to block the behavioral effects of apomorphine (0.3-32 mg/kg, s.c.), a dopaminergic agonist. A method of scoring was used which permitted a detailed analysis of the effect of apomorphine. Chronic treatment of fluphenazine completely blocked the effects of apomorphine at all 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 block the apomorphine-induced aspect of striatal DA is considerably reduced after chronic pretreatment with this drug. (Supported, in part, by USPHS grants MH20620, MH00053, and MH 63260.)
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 nonhypertensive (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·Kg⁻¹; i.p.). 5-HTP and DOPA in tissue samples were measured fluorometrically after separation on Dowex-50 W columns. 5-HTP and DOPA 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 nonhypertensive (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·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.

<table>
<thead>
<tr>
<th></th>
<th>4-wk-old</th>
<th>8-wk-old</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYP</td>
<td>1387±305</td>
<td>1377±305</td>
</tr>
<tr>
<td>P-M</td>
<td>614±47</td>
<td>113±138*</td>
</tr>
<tr>
<td>SC</td>
<td>2424±22</td>
<td>45±25*</td>
</tr>
</tbody>
</table>

*P<0.01 compared to NT control; **P<0.001 compared to NT control.

(Supported by a grant from the Illinois Heart Association.)


Radioreceptor assays have been conducted on rat whole-brain homogenate using [3H]diphenylhydantoin ([3H]DPH) and a modified filter-assay technique. These studies indicate the existence of a high-affinity, saturable binding site. Spirodillantin A, an active anticonvulsant enantiomer, competes for [3H]DPH binding whereas Spirodillantin B, the inactive stereoisomer, does not. The anticonvulsant barbiturates also show significant competition for the high-affinity binding site, but the anti-epileptic 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.

(Based on a presentation by G. McJ. from the University of British Columbia, Vancouver.)


Buckholtz and Bogdan (Biochem. Pharmac. 26, 1991, 1977) report a time-dependent inhibition of 6-MeO-THβC (6-MeO-THC) (MAO-A: 100 mg/kg ip) with a maximal inhibition (22%) by 1 hr. They also showed an inhibition (13%) of MAO-B with Ro4-4602 (800 mg·Kg⁻¹; i.p.) and brain homogenates of CF1 mice, using a column method for metabolite extraction. Meller et al. (J. Neurochem. 28, 995, 1977) showed an inhibition (18%) of MAO-A and an inhibition (32%) of MAO-B by MeO-THC (100 mg/kg) in rat hypothalamus, using an organic metabolite extraction. They also reported an IC50 of 3.7 µM for 6-MeO-THC in vitro. The present study was done to determine if different in vivo inhibition by 6-MeO-THC was due to strain difference or extraction techniques, 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 8-Phenylenediamine (PEA) for MAO-B. It was then determined that there were no differences in inhibition patterns seen with either [3H] or [14C] 5-HT as substrate for MAO-A. 6-MeO-THC 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-MeO-THC (100 mg/kg) showed a maximal inhibition by 1 hr (54%), a decline to 30% at 12 hr, and no inhibition at 24 hr. There was a maximal inhibition of MAO-B by 1 hr (168), a decline to 2% at 12 hr, and no inhibition at 24 hr. A dose study done at 1 hr. after inhibition with 6-MeO-THC to inhibit MAO-B 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 separate 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-THC showing competitive inhibition. Meller et al. had a Vm of 0.15 µM product/hr/µg protein and a Km of 11.4 µM, with 6-MeO-THC 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 3D form. The data also indicate that, at doses of 25-50 mg/kg, 6-MeO-THC 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-THC. Supported in part by P.H.S. grant MH-26712.


Several investigations have measured [3H]diazepam binding to rat brain between 4° and 37° and have shown decreased binding with increased temperature. Therefore in routine studies of [3H]diazepam binding, the incubation medium has been chilled to 4° prior to separation from the bound free [3H]diazepam. The binding kinetics of [3H]diazepam at 37° has yet been determined presumably because of the rate of dissociation of the diazepam-benzodiazepine binding site complex is too rapid. [3H]Flunitrazepam has a higher affinity for the benzodiazepine binding site than does diazepam, in addition, its rate of association with the benzodiazepine binding site is slower than that of diazepam. Consequently the rate of dissociation of [3H]flunitrazepam with the benzodiazepine binding site is considerably slower than diazepam. We have taken advantage of this property of [3H]flunitrazepam to determine its binding kinetics at varying temperatures.

In our initial studies, we observed an activation (34%) of [3H]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 [3H]flunitrazepam binding occurring at 37° was due to an increase in the number of binding sites or an affinity change, saturation curves were determined at several temperatures and the results analyzed by Scatchard analysis. [3H]Flunitrazepam binding was examined under 5 different conditions: 0° with the following: 0°, 12°, 25°, and 37°. The results of our studies showed a systematic decrease in [3H]flunitrazepam's affinity for its binding sites with increasing temperature. The Kd 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 [3H]flunitrazepam binding with increasing 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 increased temperature. Supported by USPHS grants, a Postdoctoral Clinical Pharmacology Training Grant (MH-07533) and a Research Scientist Development Award from the NIMH (MH-00095).
LACK OF TOLERANCE DEVELOPMENT IN THE RAT SUBSTANIA NIGRA FOLLOWING LONG-TERM AMPHETAMINE ADMINISTRATION. David A. Stachowiak, Robert J. Walsh, 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 firing rate of catecholaminergic neurons. Several of the physiological and behavioral effects of amphetamine show tolerance development following low doses of amphetamine. In an effort to determine whether the effects of amphetamine show a progressive augmentation following administration of a low dose of amphetamine, rats were injected daily for 4 weeks with 2.5 mg/kg of amphetamine. In experiments reported here, rats were given daily intraperitoneal d-amphetamine sulfate injections for periods of 8 (2.5 mg/kg twice daily), 5.0 mg/kg twice daily, 5.0 mg/kg once daily, or 2.5 mg/kg 5 times weekly. Intraocular injections of single neuron activity utilizing chloral hydrate anesthesia during 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 maximum 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 regimes as 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 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-01447 from the National Institute on Drug Abuse and Research Scientist Development Award E02 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 D.S.)

6-HYDROXYDOPA DEPLETES BOTH BRAIN EPINEPHRINE AND NOREPINEPHRINE: INTERACTIONS WITH ANTIDEPRESSANTS. Philip F. Fruehling and Elizabeth G. Losey.* The Upjohn Company, Kalamazoo, MI 49001. The neurotoxic agent, 6-hydroxydopamine, and its precursor 6-hydroxydopamine, 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 catecholamnergic 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 uptake of nor-epinephrine 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-hydroxydopamine provides a useful assay for identifying antidepressant drugs.

We have recently found that intravenous administration of the neurotoxic agent, 6-hydroxydopamine, to mice treated with pargyline likewise eliminated both epinephrine and norepinephrine from the brain. Pretreatment with the tricyclic antidepressant drugs (imipramine, imipramine and protriptyline) differentially blocked the effects of 6-hydroxydopamine on the effects of 6-hydroxydopamine on the effects on both epinephrine and norepinephrine, although higher doses were required to protect epinephrine. Iprindole selectively blocked epinephrine depletion while not affecting norepinephrine levels. The ability of antidepressant drugs to block the effects of 6-hydroxydopamine on catecholamines 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 imipramine, protriptyline and iprindole block the catecholamine uptake system of noradrenergic neurons, an action of antidepressants may play a role in their clinical effects, particularly in those of imipramine which does not block the uptake of other biogenic amines.
**BRAIN TRYPHTYLINE: EVIDENCE FOR EXTRACEREBRAL ORIGIN.**

Jerry J. Warsh, Donald V. Coscina, Peter W. Chen* and Ramadori D. Godse*.

Dept. of Neurochemistry and Biophysics, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada, M5T 1S8.

The presence of tryptamine (TA) in rat brain has been unequivocally demonstrated by mass spectrometric (MS) and gas chromatographic methods (Phillips et al., Can. J. Biochem. 52: 741; 1974; Warsh et al., J. Biochem. Med. 27: 47; 1977). It penetrates the blood-brain barrier (Oldendorf and Braun, Brain Research 131; 1978), brain TA may derive from peripherally tryptophan (TP) decarboxylase, 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 as before and brain TA and 5-HT were determined by GC-MS and brain TP by spectrophotofluorometry.

Co-administration of a 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.

**PHARMACOLOGICAL MANIPULATION OF BRAIN ACETYLCOLINE: DEPENDENCE ON DIETARY CHOLINE CONCENTRATION.**

Lynn Wecker and Dennis E. Schmidt*.


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 content of rat diets used by various investigators. Furthermore, acute administration of Ch to rats has been shown to modify the biochemical effects of atropine. It has been 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 (Soc. Neurosci. 399:86, 1978). There is good evidence to show that dietary conditions may be used to manipulate the Ch availability of Ch. Therefore, in several experiments, the Ch availability of Ch was manipulated by the following procedures. Male Sprague-Dawley rats (145g) were maintained for 2 weeks, with water available ad libitum, on a diet containing 11.4g free Ch/kg chow. At the end of the 2 week period, the rats were injected (ip) with saline, Ch (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 rats fed a low Ch diet was reduced to 85% of control N rats. Acute Ch administration (40 min) did not elevate hippocampal ACh levels in any dietary group. In the striatum, no changes were noted in HC rats, whereas, in N rats a significant dose-dependent increase in ACh levels was noted. These data indicate that following MAO inhibition at least a 40% decrease also occurred in brain 5-HT accumulation. From the results reported herein, it is suggested that a substantial fraction of endogenous brain TA may also be derived from the decarboxylation of TP in extracerebral pools.

**EFFECTS OF MUSCIMOL UPON THE ACTIVITY OF SUBSTANTIA NIGRA PARS RETICULATA NEURONS.**

Barbara L. Wasiczek, Joan M. Lakoski and Judith R. Walters*.

NIH, NIMH, Bethesda, MD, 20014.

GABAergic neurons that originate in the striatum and globus pallidus and terminate in the substantia nigra pars reticulata, have been postulated to tonically inhibit the activity of the nigral pars reticulata (N) GABAergic (GABA) neurons. 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 neurons in the 6-hydroxydopamine (6-OHDA) lesioned rat (Warsh et al., J. Neurochem. 27: 47; 1977). Furthermore, i.p. administration of 3.5 mg/kg muscimol produced a complete inhibition of firing of 90% of the nigral cells recorded (N=6). It was found that the drug did not inhibit firing was approximately 25 minutes (range: 5-60 min). i.v. administration of successfully increasing doses of muscimol did not produce a measurable effect on the activity of a single i.v. dose of muscimol (1.6 mg/kg) produced significant decreases in activity in 75% of cells recorded, increases in 14%, and no change in the activity of 11% (N=41). Furthermore, 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 lesions rendered non-responsive to the thermal probe of 40°C to forceps pinch were tested in the cutaneous areas of the body in those dermatomes associated with the spinal segments affected by the intrathecal baclofen. In contrast to L-baclofen, the L-isomer failed to show any antinociceptive effects even 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 produced by L-baclofen (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 response latency to the hot plate but a significant increase in the escape response latency to the cold plate. These findings are consistent with the observation that the reticulata cells are more sensitive to pontoophoresed GABA than the nigral DA cells.
THE SYNERGISTIC INTERACTION OF THREE PHARMACOLOGICALLY DISTINCT PRENATAL MATERNAL PHENOBARBITAL REDUCES CONVERSION OF TYROSINE TO CATECHOLAMINES IN BRAINS OF YOUNG OFFSPRING. Tony L. Yakeh, 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 a recent experiment that while naloxone (5 µg/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 action 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) or 5-HT (0.1 µg). These results were not sufficient 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 (0.1 µg) which alone had no 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 (0.5 µg) or MOR (1 µg) and BAC (0.1 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 that the tail flick and hot plate thresholds were elevated, the response to force cut pinches applied to the tail, 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 local action for the functional interaction of spinal modulatory systems controlling sensory throughput. This work was supported by the Mayo Foundation.


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 (62.1 ± 6.0 mg/kg), and morphine (93.5 ± 11.9 mg/kg). In lidocaine treated rats, the amplitude and rate of diaphragmatic movements and of phrenic nerve bursts gradually decreased until respiratory arrest. In contrast, respiratory rate was initially increased by cocaine and CDM. Diaphragmatic 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, continuous respiratory depression 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 lungs failed to expand for 5 sec and then were still contracted following electrical 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).


We have previously reported (Zemp et al., 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., Develop. Psychobiol. 18, 305-313, 1975; Middaugh et al., Pharmacol. 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 7 days of pregnancy. Brain concentration of TYR, DA, and NE were not altered by prenatal drug exposure; however, one hour after the injection of 3H-TYR the specific activity of 3H-TYR was significantly increased by 15-23% in the brains of 21-day-old mice exposed prenatally to the drug. The conversion of 3H-TYR to DA and NE 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 3H-TYR to DA and NE in brains of 21-day-old offspring.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dopamine (nmole/g/hr)</th>
<th>Norepinephrine (nmole/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7.54 ± .44(12)</td>
<td>2.95 ± .24(12)</td>
</tr>
<tr>
<td>Phenobarbital (20mg/kg)</td>
<td>5.41 ± .43(12)</td>
<td>2.03 ± .18(12)</td>
</tr>
<tr>
<td>Phenobarbital (40mg/kg)</td>
<td>4.74 ± .37(14)</td>
<td>1.73 ± .13(14)</td>
</tr>
</tbody>
</table>

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

In vivo studies have shown that norepinephrine doesn’t cross the blood-brain barrier (Kleiber and Axelrod, J. Biol. Chem. 237, 1838-1840, 1962). In order to elucidate the mechanism responsible for the reported observations the uptake of U norepinephrine was investigated in isolated cerebral capillaries which were previously proven to be metabolically active and suitable for such studies (Mrulja, Mrulja, Fujimoto, Klatzo and Spatz, Brain Res. 110, 361-365, 1976).

The isolated capillaries took up the U norepinephrine and the labeled substance increased with the duration of incubation (2-60 minutes). The uptake of U 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 U uptake of norepinephrine was also 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 U 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 U 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 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.

LIGHT STIMULATED INCREASE OF CHOLINE UPTAKE AND ACETYLCHOLINE SYNTHESIS IN THE TURTLE RETINA IS ASSOCIATED WITH AN INCREASE IN THE Vmx 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 Mauelshagen, 1976; Baughman and Tso, 1977). The present study concerns the effects of light stimulation on choline transport and ACh synthesis in an isolated, perfused turtle eye preparation. The ERG was monitored during the period of stimulation to confirm the viability of each eye. To control for variability in the perfusion, caused by vitreous remaining in the eyecup, the uptake of 3 H choline was measured (10 min) in the perfusate, which should block synaptic transmission and which in choline uptake, which is accompanied by an increase in ACh synthesis, caused some reduction of the ERG b-wave, choline uptake and ACh synthesis were no longer enhanced. In a flashing light experiment with 1.2 m cotinine, 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 abolished. To determine the changes in kinetic parameters underlying the increased choline uptake, choline uptake was measured in synaptosomal preparations made from retinas that had been exposed to either constant dark or flashing light. Relative to values seen after constant dark, with flashing light the low-affinity K and Vmax and the high-affinity K were 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 Vmx of the high-affinity choline uptake system. (Supported by NIH Grants EY01995 and EY00082.)


Analysis of conductance fluctuations induced on peripheral vertebrate and cerebellar protoplasmic (EY 02423 and NS 13224).

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, 1977) 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 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., I. A. L. and K. L. 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 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 dianilinobenzidine 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 the remaining half of the HRP reaction products. Results similar to that seen in 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 Houston Chorea Foundation and the NIH (EY 02423 and NS 13224).

Adenosine (ADO) causes a 200% increase in intracellular 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 cardiac cortex. Two of these analogs 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'-methyl-ADO, which has a Ki of 0.4 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 E1 (PGE1) as well as to 10 µM and 1 µM 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. Ki values for some important xanthine derivatives were:

<table>
<thead>
<tr>
<th>Ki (µM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline</td>
<td>13</td>
</tr>
<tr>
<td>7-(2-chloroethyl)theophylline</td>
<td>5</td>
</tr>
<tr>
<td>8-p-bromophenyltheophylline</td>
<td>0.05</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.3</td>
</tr>
<tr>
<td>1,3-dimethylxanthine</td>
<td>5</td>
</tr>
<tr>
<td>7-(2-chloroethyl)adenine</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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.)


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 dopamine agonist-like action during acute treatment, i.e. 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 (pCPA) (50 mg/kg), a metabolite of pCPA, was found to produce in mice head weaving, tremor, hyperactivity, Straub tail, hind leg abduction and salivation; a syndrome characteristic of serotonin receptor stimulation (Jacobs, B.L., 1976). The intensity and duration of this pCPA-induced "serotonin" syndrome was enhanced by the monoamine oxidase inhibitor, pargyline (10 mg/kg) and Org 6582 (10 mg/kg), which are serotonin uptake blockers. 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 pCPA (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 (100 mg/kg, 24 hours prior to pCPA) 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.

The biochemical data suggest that pCPA is taken up by the same neuronal transport process as serotonin and causes endogenous serotonin to be released. However, pCPA is probably also a direct serotonin receptor agonist, since it can produce the "serotonin" syndrome without pretreatment with fluoxetine, Org 6582 or pCPA. The previously reported paradoxical early effects of pCPA may be due to the action of its metabolite pCPA.

(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. Judy Patricia Corey* and Carl R. Wasse. 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). Schwarz 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 "sustained" effect was demonstrated by Bird et al. (1978) to demonstrate glutamate's role as a primary afferent neurotransmitter in the rostral AVH of the cochlear nucleus. The observed effects have 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 by the injection. The olfactory bulb is a 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.

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 cranialotomy 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 via a hydraulic microdrive. Local injection of morphine (10-4 M) into the CN at a volume of 1 ul were made by means of a stereotaxically placed 27-gauge syringe needle attached to a microinjection-device. During the initial 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 morphine (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 HCI by Endo laboratories used in the present study.)
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 mitochondria; however, the mechanism of transfer of the active acetyl units across the mitochondrial membranes is a matter of controversy. Quastel (Cholinergic Mechanisms and Psychopharmacology) and colleagues et al. (Biochemistry 59, 197) have suggested that the active acetyl groups for acetylcholine synthesis may be generated in the cytoplasm. These suggestions led us to reexamine 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 in a high potassium (110 mM) Krebs-Ringer phosphate buffer with 1.25 mM glucose, 50 mM choline and 40 mM paraoxon.

In the first series of experiments, the mass of acetylcholine and the incorporation of [1-14C]choline into acetylcholine were determined. Thus, we could determine the specific activity (DPM/nanomole) of the synthesized acetylcholine. Addition of K+L+ of glucose, acetylphosphate was unable to stimulate synthesis of acetylcholine (4 counts). The lack of incorporation was not due to lack of incorporation of acetylcholine, since greater than 90% of Acetate (10 mM) itself had no effect on acetylcholine specific activity. Although synthesis of acetylcholine as measured by GC-MS was also determined. Thus, we could determine the specific activity (DPM/nanomole) of the synthesized acetylcholine. Addition of K+L+ of glucose, acetylphosphate was unable to stimulate synthesis of acetylcholine (4 counts). The lack of incorporation was not due to lack of incorporation of acetylcholine, since greater than 90% of Acetate (10 mM) itself had no effect on acetylcholine specific activity. Although synthesis of acetylcholine as measured by GC-MS was also determined. Thus, we could determine the acetylcholine specific activity was in part due to the addition of the excess K+ and L+ which caused inhibition when added as the chloride salts (31.8 ± 2.7% of control with 100 mM). The specific activity was increased by their addition (13.7 ± 15.2%).

In order to determine if there was a direct transfer of acetyl groups from acetylphosphate to acetylcholine, synaptosomes were incubated with [1-14C]acetylphosphate synthesized from [1-14C]acetate. The purity was determined by enzymatic assay to be 89.5%/1%. Spectrophotometry showed it to be identical to commercial acetylphosphate. Although large amounts (38 X 10^6 CM) of [1-14C]acetylphosphate were incubated with synaptosomes, no radioactivity above non-incubated control values were found in acetylcholine (6 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. Acetyl (10 µM) itself had no effect on acetylcholine specific activity (97.5 ± 2.7% of control). Furthermore, in the absence of glucose, acetylphosphate was unable to stimulate synthesis of acetylcholine and non-incubated controls. Thus, acetylphosphate does not appear to be an intermediate in acetylcholine synthesis.

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) in rats enarar to other therapies in 9 of 20 patients (none of whom responded to placebo). The naturally-occurring dietary Ch source is lecithin (Lec) (Phosphatidylcholine). In large amounts it 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 does choline or lecithin (Table 1). 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. The abnormal passage of sucrose into the ischemic hemisphere was evident at all periods studied whereas abnormal elevation of Ch 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 neuritic 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 sucrose 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.

**Table 1**

<table>
<thead>
<tr>
<th>Subject Lecithin</th>
<th>Choline Movement</th>
<th>Improvement</th>
<th>Serum Choline</th>
<th>Percent Choline</th>
<th>Before During</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leech</td>
<td>60</td>
<td>1.8</td>
<td>jaw</td>
<td>90</td>
<td>12.2</td>
</tr>
<tr>
<td>Lec</td>
<td>80</td>
<td>2.4</td>
<td>facial</td>
<td>75</td>
<td>7.5</td>
</tr>
<tr>
<td>Lec</td>
<td>30</td>
<td>4.8</td>
<td>tongue</td>
<td>86</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Lec was as effective as Leech in reducing choreic movements, but, unfortunately, did not produce the flabby odor that accompanies Ch ingestion. Leech 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.)


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 NE were served as tracers and 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 30-15 hour interval following release of occlusion. Quantitative measurements of NE and NE choline revealed that abnormally high passage of sucrose into the ischemic hemisphere was evident at all periods studied whereas abnormal elevation of NE 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 neuritic 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 sucrose 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.


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) in rats enarar to other therapies in 9 of 20 patients (none of whom responded to placebo).

The naturally-occurring dietary Ch source is lecithin (Lec) (Phosphatidylcholine). In large amounts it 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 does choline or lecithin (Table 1). The lack of incorporation was not due to hydrolysis of added acetylcholine, since greater than 90% of the acetylphosphate remained at the end of the incubation. Acetyl (10 µM) itself had no effect on acetylcholine specific activity (97.5 ± 2.7% of control). Furthermore, in the absence of glucose, acetylphosphate was unable to stimulate synthesis of acetylcholine and non-incubated controls. Thus, acetylphosphate does not appear to be an intermediate in acetylcholine synthesis.


The effects of two inhibitors of y-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 Okada, et al. (Exp. Br. Res. 13: 514, 1971). Time course of the dypropilacate (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 (125% 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. In doses of 200, 300 and 400 mg/kg, 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.
The internal pH of isolated serotonin granules was measured by [14C]-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 press cell, and fractionation on a sucrose-Ficoll-D2O gradient in order to preserve isotonicity. When [14C]-methylamine was added to a granular preparation suspended in 0.3 N sucrose-30 m M 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 alkalization of the intragranular space. The addition of 400 µM CaCl2 and A23187, an ionophore which transports Ca2+ in exchange for protons in a ratio of 1:2, resulted in a ΔpH of 0.65. Addition of 30 mM NaCl which is thought to permeate biological membranes as NH4+ 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 ΔpH such that at pH 7.1, 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 ΔpH is not due to the establishment of a Donnan equilibrium. The ΔpH-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 the ΔpH units; and the addition of 33 mM serotonin to 0.25 pH units. When serotonin granule membranes form by hypoosmotic lysis of the granules were used, the ΔpH 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 amine, and that high concentrations can permeate the membrane in the uncharged form are similar to previous studies of another amine containing the granules were utilized. Data from pig platelet serotonin granules catalyze the synthesis of the neurotransmitter acetylcholine (ACh) in the cholinergic neurons. There is no satisfactory histochemical method for the localization of ACh at the cellular level. The localization of acetylcholinesterase and the published histochemical methods for the localization of ACh 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 in 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 was also found in 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 cerebellums. 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. YS-11087.

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 buccal lingual 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.

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 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 2 mm 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 40µ of protein) were homogenized in a 0.6ml volume. Ten lambda aliquots were assayed using a method similar to that of Albert and Brady (JBC(1959) 234:926) in which enzymatic activity was assessed by the amount of CO2 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 much as a five fold range from 0-45 munits/mg/protein in the outermost plexiform layer to 400 munits/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 lends to the further suggestion whether this activity is attributable to cells intrinsic to this layer or to entering processes.
SOCIETY FOR NEUROSCIENCE

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 resis­
tance, and lack of response to dorsal and ventral root or intra­
cellular 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 pushpull 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.

1417

OPIATE AND CHOLINERGIC INDUCED CHANGES IN MEMBRANE POTENTIAL MON­
ITORED BIOCHEMICALLY IN NEUROBLASTOMA X GLIOMA HYBRID TISSUE CUL­
TURE 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 (ΔΨ) of -40 to -60 mV as de­
termined by direct electrophysiological measurements. In an at­
tempt to develop a biochemical method for monitoring changes in
ΔΨ in populations of cultured neuronal cells, accumulation of the
permeant lipophilic cation [3H ]tetraphenylphosphonium (TPP+)
[bromide salt] has been studied. TPP+ is accumulated against a
concentration gradient by NG108-15 cells suspended in physiologi­
cal 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 ΔΨ in NG108-15, the difference in TPP+ accumulation
in high Na+ versus high K+ has been taken as an index of ΔΨ, and
using the Nernst equation (ΔΨ = 61 log [TPP+ ]in/[TPP+ ]out), a
resting potential of ~ -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 func­
tion of cell number and TPP+ concentration (i.e. ΔΨ is indepen­
dent of TPP+ concentration up to 26 µM). Moreover, TPP+ accumu­
lation is decreased or totally abolished by carbonylcyanide mchlorophenylhydrazone, dinitrophenol or ouabain. In contrast,
the ionophore monensin which catalyzes electroneutral transmem­
brane exchange of Na+ and H+ causes an increase in TPP+ accumula­
tion. Alterations in TPP+ accumulation are also induced by addi­
tion 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-ala2-met5-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 nalo­
xone. The data demonstrate clearly that (i) membrane depolariza­
tion and hyperpolarization can be monitored biochemically in cul­
tured cell populations; and (ii) in NG108-15 cells, hyperpolari­
zation is induced by agonist occupany of either opiate or
cholinergic receptors.

1 4 1 6 INHIBITION OF HIGH AFFINITY GLUTAMATE ACCUMULATION BY KAINIC
ACID - A KINETIC AND PHARMACOLOGICAL STUDY. John Lehmann, E. G .
try, University of British Columbia, Vancouver, B.C.
V6T IW5,
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 Ki
was 7.5 x 10-4M and was apparently non-competitive with glutamate
In contrast, both glutamate and aspartate were competitive inhi­
bitors, with Ki of 3.7 and 2.3 x 10-6M 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 inhibit­
ion indicates that kainic acid exerts its action at a site dis­
tinct 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, re­
duced control uptake velocity and tended to enhance KA’s inhibit­
ion.
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 intra­
cerebral injections of cytotoxic doses of KA.
Supported by the Medical Research Council.

1418

INTERACTION OF GAHA 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, 3 1,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 dec­
reased 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 neurolep­
tics, the DA neurons became much more susceptible to GABArelated drugs. Thus, haloperidol-induced catalepsy was poten­
tiated 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 turn­
over 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 path­
way 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 nigrostriatal DA pathway ; and ii) behavioural effects of direct
stimulation of DA receptors by apomorphine are not overcome by
SL or M.

446


POSTSYNAPTIC PHARMACOLOGY OF CEREBELLAR NEURONS IN CELL CULTURE. R. L. MacDonald, G. Moogan*, 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-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, intracellular recordings were obtained from the largest neurons (some diameter 15 µm). These neurons were polyglial in shape with 1-5 dendritic shafts and fine dendritic arborizations, and appeared, on morphological grounds, to be the in vitro equivalent of Purkinje cells. All neurons studied with membrane potentials between -50 and -40 mV (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, was involved in the response. Moreover, at low currents augmentation of GLU responses only at high currents (n=15). Responses were also elicited by NE (n=7), DA (n=8) and 5-HT (n=8).

GLU reversibly increased membrane conductance and was depolarizing with reversal potentials between 0 and -10 mV (n=4). There was no unitary sensitivity to GLU with high sensitivity or occurring only on the depolarizing little sensitivity to GLU was present on the soma. GABA also reversibly increased membrane conductance but was hyperpolarizing with reversal potentials (n=6) between -50 and -40 mV (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=4) and GABA of about 2.0 (n=7), suggesting that GABA interacts with its receptor in a cooperative manner. Although having little direct action, ASP was a potent modulator of GLU responses (n=6). At low preejection levels and shifted the GLU OR curve to the left while higher currents drastically reduced GLU responses. NE and DA were both inhibitory and decreased or abolished activity entirely. The 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 and further suggests that this culture system may be useful in the study of cerebellar neurotransmitter mechanisms.


Intracellular recordings were made from giant reticulospinal neurons (MiUller cells) in the isolated brain of lamprey, and the physiological characteristics of the inhibitory synaptic potential (ipsp) evoked by ipsilateral vestibular nerve stimulation were compared with those of the hyperpolarization resulting from the excitatory action of glycine or α-amino butyric acid (GABA). The reversal potentials of the ipsp, the glycine response, and the GABA response were identical, averaging -63 mV, which is the usual -70 mV resting potential of the cells. To determine if a change in C1 conductance was involved in the response, the C1 equilibrium potential was changed intracellularly or by lowering extracellular C1. Both manipulations shifted the reversal potentials for glycine, GABA and the idsp in a positive direction by identical amounts. Alterations in extracellular K produced identical changes in the reversal potentials for the drugs and the idsp, 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 C1 concentration. Picricotoin bicuculline at a concentration of 20 µM abolished the response to GABA but had negligible effects on the idsp or the response to glycine. In contrast, 20 µM strychnine abolished both the idsp 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 squedular region (the I cells), this was accompanied by a parallel reduction in the amplitude of the idsp. However, in some Müller cells in the midbrain (the IIcells), 1 µM strychnine, although rapidly abolishing the glycine response, had no effect on the idsp, which required approximately 5 µM strychnine before it was substantially reduced. This suggests that lamprey Müller cells glycine is a better candidate for the inhibitory transmitter than is GABA, particularly so for those cells (the II cells) in which the sensitivity of the idsp and the glycine response to strychnine was identical. (Supported by NIH grants NS 09661 and 09660.)

Adenosine (Ado) and its non-metabolizable analog 2-Chloroadenosine (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 dogma (DA) stimulation of AC activity (Neuroscience Abst. 311, 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 adenosine nucleotides on cortical neurons. Adenosine, AMP-PNP, B, γ-methylene ATP, and 5'-AMP are approximately equipotent in stimulating AC activity but less effective than 2-Cl-Ado. The potent methylxanthines, theophylline and theophyllinol, 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 endogenously released DA from synaptosomes. The magnitude of the inhibition by 2-Cl-Ado of the depolarization (KCI)-induced release of DA is dependent on the strength of the depolarizing stimulus, i.e., KCl concentration. The depolarization (KCI)-induced release of DA is also observed when the releasing stimulus is exposure to various amounts of endogenously released Ado. On reagent of D-aminoadipate. These results suggest the presence of endogenously released Ado which is continuously controlling DA release. The depolarization (KCI)-induced release of 3H-DA with the greatest effect at the lower KCl concentrations. The 2-Chloroadenosine modulates the release of endogenous Ado which is continuously controlling DA release from nerve endings. 2-Chloroadenosine has no effect on the affinity of 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-21357 to E.K.M.

A COMPARISON OF THE EXCITATORY EFFECTS OF L-ASPARTATE AND L-GLUTAMATE ON PURINJKE 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 46250.

L-aspartate (Asp) and L-glutamate (Glu) were administered iontophoretically onto Purkinje cells and other cerebellar neurons of phosphate-anesthetized rats. Both 50 Hz (0.5 M, pH 8.5) pulses stimulated an increase of spike frequency in all cells tested. The excitatory effects of both agents were characterized by an initial slow increase (< 2 sec) 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 potency of aspartate, 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 ED50) 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 antagonists showed glutamate acid diethylster 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 Grant GM06959 from NHM and NS13925 from NIMH).


The in vitro uptake of [35S]cytstine 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° 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 mechanism, and the double-reciprocal plot of the cysteine uptake suggests dual affinity system with the Km values for the high affinity uptake of about 16.5 x 10^-4 M and for the low affinity uptake of about 4.0 x 10^-3 M. This transport was also found significantly inhibited by ouabain, a potent inhibitor of the Na-K dependent ATPase and other metabolic inhibitors.


The olfactory bulb was dissected into several discrete layers useful for studying the distribution of amino compounds and [3H] ligand binding sites. Dogs under barbiturate anesthesia were exsanguinated and the olfactory bulbs removed into liquid N2 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)-afferent and efferent axons. Tissue was either deproteinized and analyzed for amino compounds, usingicar.41) or else membrane fractions were prepared for [3H]ligand binding studies. Levels of several amino compounds and the binding of nine [3H]ligands were measured. Binding of [3H]carnosine (carn) was highest in GL and lower in F, M-G and W. [3H]Dihydromorphine binding was high in both F and M-G, but was much lower in GL and W. [3H]Diazepam (Dz) binding was primarily localized in M-G, while [3H]flumazenil (Mu) and [3H]muscimol (Mu) binding were predominant in GL and M-G. [3H]CB binding was uniformly distributed. α- and β-adrenergic binding was distributed differentially 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 tyro levels were high in M-G and low in F, GL and W. The levels of tau, β-ala, glut, asp, ser, gly, ala and phen showed no significant differences among the four layers. The distribution of carn and its binding site support previous reports from this laboratory implicating this peptide as a neuroactive at the glomerular level. While the localization of GABA and Mu binding is consistent with data from other laboratories associating it with granule cell function, the significance of high levels of both Asp and Glu 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.

<table>
<thead>
<tr>
<th>µmol compound/tissue</th>
<th>fmol ligand bound/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer</td>
<td>Carn</td>
</tr>
<tr>
<td>F</td>
<td>1.27</td>
</tr>
<tr>
<td>CL</td>
<td>0.95</td>
</tr>
<tr>
<td>M-G</td>
<td>0.34</td>
</tr>
<tr>
<td>W</td>
<td>0</td>
</tr>
</tbody>
</table>

448
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.


This work was supported by NSF PCM 710134 and USPH AM 12768.


VOLTAGE-DEPENDENT EXCITATORY RESPONSE TO SEROTONIN IN APYSLA. T. C. Pellmar and D. O. Carpenter. AFRI, NIMH, Bethesda, Maryland 20014.

A slow voltage-dependent excitatory response to lontophoretic 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: gluconate prolongs the response to serotonin; succrose 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.

Voltage-dependent excitatory response to serotonin in *Aplysia*. 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: gluconate prolongs the response to serotonin; succrose 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 evoked; but such a current carried by a current carried by a potassium ion has a possibility. Alterations of extracellular calcium may not alter the calcium gradient sufficiently to modify such current. The above data seems more consistent with a decrease in a potassium conductance. Yet, it is disturbing that moderate changes in potassium concentration have minimal effects.

(20014)
1431 and/or trauma may affect neurologic recovery, and should be an inverse correlation between neurologic score and levels of regional DOPAC and HVA, with normal levels in survivors showed an inverse correlation between neurologic score and regions with x-irradiation. Treatment with x-irradiation caused a significant (P<0.01) 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 which had been exposed to x-irradiation treatment on days 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<0.01) 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 which had been exposed to x-irradiation treatment on days 8 to 15 following birth. The level 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. The level of GABA in the cortex was increased (P<0.02) by 15% with x-irradiation. The level of GABA in the P1 fraction was the same for both groups. The uptake of [3H]Glu and [3H]Asp at a concentration of 1 µM was approximately 20% lower in the P2 fraction from the x-irradiated group than from the control animals whereas the uptake of [3H]GABA was nearly the same in both groups. More detailed kinetic analysis of [3H]Glu uptake revealed that the Km value was not significantly changed but the Vmax value was significantly (P<0.01) lower (by 20%) for the x-irradiated and normal group. 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 change was observed in the concentration of HIAA observed in the brains of these trained rats. These results suggest that methadone conditioning of striatal HVA was not accompanied by a conditioned reflex change in the basal forebrain. 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.

(Supported by Grant NS13042, National Institutes of Health)

The neurotransmitter serotonin (5-HT) is a potent vasoressor substance whose presence within nerve terminals of brain intra-parenchymal blood vessels has been recently suggested by autoradiographic and histofluorescent studies. Serotonergic 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 microsieves, after meninges and choroid plexus 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 implanted canula. Rats were killed 5-20 min later, brains were dissected and brain regions analyzed for central CA projections. (Supported by USPHS Grants MH-11191; and 5-K02-10562.)


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 method using a temperature-programmed capillary column. A 24 hour fast increased the concentration of both metabolites in the plasma and CSF. Mean (± SE) plasma HVA levels were 7.3 ± 0.8 ng/ml, 2 h after the injection of haloperidol (0.5 mg/kg, i.m.) and 7.9 ± 0.9 ng/ml, 4 h after the injection. HVA was isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and was measured fluorometrically. The metabolites of homovanillic acid (HVA) were isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and were measured fluorometrically. The metabolites of homovanillic acid (HVA) were isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and were measured fluorometrically. The metabolites of homovanillic acid (HVA) were isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and were measured fluorometrically. The metabolites of homovanillic acid (HVA) were isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and were measured fluorometrically. The metabolites of homovanillic acid (HVA) were isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and were measured fluorometrically. The metabolites of homovanillic acid (HVA) were isolated from the plasma of rhesus monkeys with a strong affinity for the probenecid probe and were measured fluorometrically.
Purification of L-glutamate decarboxylase from catfish brain.

Y. Y. Thomas Su*, J.-Y. Wu and Dominic M. K. Lam*
(Spon: E. Peck) Colby Eye Institute and Department of
Cell Biology, Baylor College of Medicine, Houston, Texas 77030

L-Glutamate decarboxylase (GAD) has been purified from mouse brain 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., 1977). However, the immunological specificity of the antibody against this enzyme does not cross-react with the GAD from neural tissues of fish. We have therefore begun to purify GAD from catfish brain in an attempt to obtain specific antibodies with a view to localizing GAD in GABAergic neurons in teleost retinas. GAD was extracted from catfish brain by homogenization in the tissue-water-sodium pyrophosphate buffer, 1 mM EDTA, 1 mM EGTA and 1 mM reduced glutathione, pH 7.2. About 70% of GAD activity was recovered in the supernatant of 100,000 × g. 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 immunohistochemical and histochemical studies of the GABA system in teleost retinas.

Supported in part by the Retina Research Foundation (Houston), NIH grants ET 02423 and NS 13224 and a grant from Huntington's Chorea Foundation in memory of Mrs. Ruth Berman.

Pitfalls in the use of the automated amino acid analyzer for the quantitation of some acidic amino acids in brain tissues.

K. H. Tachibana, C. E. Melo, A. M. Balkin and C. F. Balkin
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 common and undisputed premise: amino acids retain their identity during homogenization, purification and hydrolysis of the sample. 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 containing 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-8 resin, AG1-X8 resin and paper chromatographic techniques. In the last system, a butanol/acetic acid/water (12:3.5:2, v/v) solvent separated the unknown compound from taurine. Upon hydrolysis, large amounts of ethanolamine and phosphate were detected. However, the unknown compound was not phospho­
rorylatable to taurine. It has the unusual structure of glycero­
phosphorylhexanamine. Several investigators have reported a loss of "taurine" upon hydrolysis of mammalian brain pre­
parations (Hussain, E. and Marcuccio, F., in Amino Acid Pools, ed. by J.T. Holden, Elsevier Publ. Co., New York, p. 486-492, 1972; Lähdesmäki, P., Karpinnen, A., Saarni, H. and Winter, R., Brain Res. 159, 295-308, 1977). All of these findings emphasize 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 additional variable which may co­
chrmatograph with aspartate has also been detected. Tau­
rine levels in brain tissues have been linked to the con­
trolment of convulsant drug actions and the catalytic role of glycero­
phosphorylhexanamine in the central nervous system is not well es­

tablished. Experimental and clinical data for taurine levels of­
ten have been determined with the help of automatic amino acid analyzers. Some of this data in the literature deserves to be re­
evaluated.

The Relationship between calcium-dependent and independent release of [3H]GABA from cortical slices evoked by picrotoxin, veratri­
der 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 Ca2+; raising the possibility that Ca2+-dependent and independent release may occur from two distinct pools. To study this possibility the time course of the evoked release of [3H]GABA was followed during 48 min superfusion with either 50 mM K+ or 50 µM veratridine in a solution which had been incubated for 15 min with 1 µM [3H]GABA and 10 µM anticonvulsant. 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. How­
ever, while release by K+ was decreased by 66% when Ca2+ was omitted, the release by veratridine was unchanged in the absence of Ca2+. 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+, Ca2+ was omitted. Omission of Ca2+ following the initial superfusion with 50 mM K+ and Ca2+, 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 Ca2+. Release induced by 24 min electrical stimu­lation (64 Hz, alternating polarity) was delayed by the omission of Ca2+.

Cage convulsant drugs inhibit picrotoxin binding to mammalian brain membranes.

Maharaj K. Ticku and Richard W. Olsen, Depar­t. of Biochemistry, Univ. of California, Riverside, CA 92521.

Bicyclic phosphates [R{(U-C)O}{CO}_2]{R'}, bicyclic orthoaracnoic acids [R'COO{R''}]{S'}-esters, and dithiopyrophosphate [S']-esters inhibit [3H]GABA binding in the superfusion of rat brain slices, which causes a 30% decrease of [3H]GABA and [3H]GABA binding to rat brain membranes (Su, et al., Fed. Proc. 36, 1977). Inhibitory effects of bicyclic phosphates and dithiopyrophosphate derivatives are more potent than those with a larger R' substituent (e.g. -COO{R''} {S'}-esters). However, bicyclic phosphates and dithiopyrophosphate derivatives are less potent than those with a smaller R' substituent (e.g. -COO{R''} {S'}-esters). The bicyclic phosphates and dithiopyrophosphate derivatives also inhibited [3H]GABA binding to rat brain membranes, which shows that these compounds inhibit [3H]GABA binding to the receptor site. Bicyclic phosphates are more potent than the corresponding dithiopyrophosphate derivatives. These results suggest that [3H]GABA binding to the receptor site is inhibited by bicyclic phosphates and dithiopyrophosphate derivatives.
The relationship between choline transport and acetylcholine release. Ken Veca (SPOR: I, Shaskey). Physiology Section, Biomedical Sciences Group, Univ. of Connecticut, Storrs, CT 06268

The chick ciliary nerve-iris preparation was used to investigate the relationship between endogenous, 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 γ-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 uM), 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 Li+. Depolarization in Ca²⁺-free, 10 mM Mg²⁺ medium fails to significantly reduce endogenous ACH levels. The conditioning depolarization in the presence of Ca²⁺ increased the integral of the initial velocity of γ-ACH release, evaluated over a 2 min interval, several-fold relative to control. After a conditioning depolarization, extended preloading with γ-ACH resulted in an increase in the initial release of γ-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 γ-Ch decreased the rate of decline of γ-ACH release with successive depolarizations. A comparison of the rate of release of γ-ACH with the rate of decline of endogenous ACh suggests that newly synthesized (radiolabelled) ACh is preferentially released relative to the other 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.


Clinical and pharmacological studies have demonstrated a relationship between 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 was evident. The mere presence of a several hundred fold excess of native toxin. 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 excitatory input. In undamaged cells (resting potentials about -70 mV) it was not possible to pass enough current to reverse the epsp and glutamate response, and their reversal potentials were essentially null. In damaged cells (resting potentials about -70 mV) the glutamate response was enhanced by extrapolation. The values obtained in one such experiment were 28 mV (epsp) ± 33 mV (glutamate response). The errors involved in extrapolation were ± 2 µg to ± 8 µg and glutamate response in cells which had been damaged by extracellular NaCl (150 mV) ± 55 mV) by implication with a low-resistance microelectrode. In these experiments the reversal potentials of 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 of its normal value brought the glutamate potential by about -14 mV, indicating that an increased Na conductance was involved in the depolarization. Injection of CI intracellularly had no effects on the neural reversal potentials. Since the epsp-drug reversal potential was negative to the Na equilibrium potential and CI was not involved in the response, it appears that there is also an increase in K conductance, although this was not investigated. The rationale is a candidate for the excitatory transmitter onto Müller cells. (Supported by NIH grants NS 09661 and 09660.)
MODIFICATION OF ACETYLCHOLINE TURNOVER RATE IN SELECTED BRAIN REGIONS: EFFECT OF PARASYMPATHOLYTICS. P.L. Wood* and D.L. Cheney

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 and provides an action on presynaptic cholinergic receptors but may produce its effects through a feedback control via the septum.
PAIN


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 to establish the effects of morphine and other analgesic agents on the trigeminal system. The effectiveness of morphine and other analgesic agents in the trigeminal system was determined using a microsyringe fitted with a 3% gauge needle, 0.1 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 hind leg and by application of noxious heat to the palm of that foot. Also 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. Furthermore it is concluded that this analgesic effect is mediated by a morphine like compound acting at sites other than the nucleus raphe magnus.


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 antemio-

1462 DISASSOClation 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 antemio-

bic glucose analogue, displayed time-dependent analgesia and time-dependent elevations in both food intake and pain thresholds. The former seems likely to be mediated by acute cellular glucos-

piration 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 abbre-

viate 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. Rats above 2-DG-induced hyperphagic effects. By contrast, rats continued to induce significant increases in food intake. During recovery pain thresholds remained normal but food intake showed some increases, post-2-DG. Hence, the present data demonstrate that the analgesic and hyperphagic properties of 2-DG are experimentally dissoci-

able, and offer further evidence that 2-DG may increase pain thresholds via a 2-DG-induced activation of pain-inhibitory systems. (Supported by NIH Grant #NS 14449 and N.Y.S. Health Research Council Grants #365 and #922.)
LATERAL HYPOTHALAMIC MODULATION OF ESCAPE FROM STIMULATION OF NUCLEUS GIGANTOCELLULARIS IN THE RAT. Kenneth D. Carr and Edgar R. Conant.

When the intensity of LH-pulse trains was above stimulation-threshold and fed back for self-stimulation, the animal was taught to press a bar-pressing through electrodes implanted in the same region of the dorsal raphe nucleus. These data are consistent with the hypothesis that the ventrolateral central grey contains analgesia substrates which operate differently on continuous pain than on transient thermal pain. In a monkey with a restricted injection, the labelled areas of the midbrain were found in the parabrachial nucleus of V, but not the superior colliculus. In a monkey with a diffuse injection, spread to the auditory relay nuclei in the medial pons, inferior colliculus, the midline dorsal raphe nucleus and also the red nucleus and mesencephalic nucleus of V. This is concluded that there are substantial projections to the midbrain reticular formation from the midbrain RF and periaqueductal grey.


The injection was centered over the m1c in 3 cats and 1 monkey. These animals had labelled cells bilaterally in the midbrain RF, periaqueductal grey and the deeper layers of the superior colliculus. The RF and periaqueductal grey label was heaviest ipsilaterally and the superior colliculus label contralaterally. An injection ventromedial to the m1c in 1 monkey, with spread into the medial inferior olivary nucleus, labelled cells on the same side. A restricted injection in the midline dorsal raphe nucleus resulted 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 02956 and by NW postdoctoral fellowship NS 05078 (to L.H.R.).

NARCOTIC ANALGESIA: CHANGES IN NEURAL ACTIVITY RECORDED FROM PERIAQUEDUCTAL GREY MATTER OF RAT BRAIN. Hugh E. Criswell and Frederick B. Rogers. Dept. Psychol., Williams College, Williamstown, MA 01267.

458

Electrodes were implanted in a region extending from 0-1 mm caudal to the intraluminal line, 0-1.25 mm lateral to the midline, and 0-1.6 mm ventral to the center of the aqueduct.

Two separate groups of rats (n=10) were taught to self-stimulate (bar-pressing) through electrodes implanted in the same superficial layers, and 3 days, the animals were perfused with Ringers followed by 2.5% glutaraldehyde. The perfused brains were sectioned at 250 µm and reacted with diaminobenzidine and tetramethylbenzidine. Postfixation, the brains were cryoprotected, sectioned, then stained with cresyl violet for orientation. 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 overlapping systems mediate the effects of analgesia on different kinds of pain. This suggests either a common neural system for analgesia and self-stimulation, or two systems inextricably mixed in this pain-induced behavior.

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 an approach involving 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 3-10 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 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 prostanoid 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 brain. (Supported in part by a grant from Hoffman-La Roche and by NIH - GSB funds.)

SUPERIORITY OF INTERMITTENT VERSUS DIRECT CURRENT ELECTROANALGESIA AT HIGH CURRENT LEVELS. R. Wayne Fields, Robert P. O'Donnell, Richard T. Hatfield, and 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 pulp (Fields et al., Exp. Neurol. 51:293-303, 1976) and that trains of rectangular pulses at 1000 pps with similar peak intensities but with duty cycles as low as 10% are equally efficacious (Fields et al., Exp. Neurol. 53:366-398, 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 paper was expanded upon our previously described methods for recording thresholds of pulp-drawn 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 100 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 applied to the 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-drawn units progressively rose along essentially identical curves with increasing EA intensity, reaching 1000% of control thresholds at 4000 µA. Pulpal thresholds also progressively rose to 1000% of control using pdc EA but the hypoexcitability was less pronounced than that of the other waveforms for EA intensities above 400 µA. Pulpal thresholds reached 1500% 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)

STIMULATION OF THE PERIAQUEDUCTAL GRAY (PAG) ALTERS THE M PEAKS OF THE THALAMIC PAIN CODE. F. Campos. Department of Physiology, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

As reported in Brain Research 123:925, 1976, thalamic neurons which relay noxious stimulation are excited in a temporal pattern characterized by several activity peaks on spike density histograms. PAG stimulation at high intensities results in excitation of stimulated neurons; late spike potentials form M peaks that are related to the modality of theafferent activity. Further, the M peaks are produced by excitation of a positive feedback loop between the thalamic 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 the 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 diminished by changing the parameters of the PAG train (from 0.5 to 3.0 sec at 50/sec for 200 msecs per 400 msecs). These results suggest that different modes of PAG stimulation could make an animal either more or less susceptible to pain. Furthermore, 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 periaqueductal gray (Campos, Brain Res. 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. 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 in the perception of pain (Aided by grant NS-03266 from NINCDS).
Tooth pulp primary afferent depolarization (PAD) and naloxone: Are presynaptic opiate receptors involved? J. B. Rob, J. O. Dostrovsky, and B. J. Seagle, Fac. of Dentistry and Dept. of Physiology (J.O.B.), Univ. of Toronto, Canada.

Recent studies suggest the existence of opiate receptors on primary afferent neurons. 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 opioid-induced analgesia, depress trigeminal (V) brainstem responses to tooth pulp (TP) and noxious facial stimuli, we compared the effect of fentanyl (11 mg/kg diazepam administered to mask the different subjective effects produced by subsequent administration of either 0.66 µg/kg fentanyl or saline placebo. The diazepam and fentanyl combination significantly reduced sensitivity (P=8.05, p<.05) but not the unpleasantness of the sensations evoked by these stimuli. Diazepam with saline significantly reduced unpleasantness (P=0.21, p<.05) but not sensitivity. 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.

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 of 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.

In this study we sought to determine if the TP PAD produced by N. oralis or N. caudalis and/or oralis are subject to primary afferent depolarization from PAG, NRM, VPM, and facial-skin sites; the ineffective nature of naloxone to reverse the PAD effect from N. caudalis and/or oralis that presynaptic opiate receptors might not be involved in the PAD.

(Supported by the Canadian MRC and NIH grant F1-ROL-DE04786-01.)


The region of the periaqueductal gray matter (PAG) in the rat has a high concentration of opioid analgesics and it is well established that injection of small amounts of morphine into this region induces analgesia. We have been studying the effects of antibodies to ganglioside on synaptically evoked responses in the rat midbrain (e.g., weak, moderate, strong) and unpleasantness (e.g., unpleasant, moderate, strong) of the sensory intensity (Sessle et al., Can. J. Physiol. Pharmacol. 54: 107-118, 1976), we tested for a PAD contribution to the depression. We found that all of 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 PAD produced by N. oralis and/or oralis is subject to primary afferent depolarization from PAG, NRM, VPM, and facial-skin sites; the ineffective nature of naloxone to reverse the PAD effect from N. caudalis and/or oralis that presynaptic opiate receptors might not be involved in the PAD.

(Supported by the Canadian MRC and NIH grant F1-ROL-DE04786-01.)


The region of the periaqueductal gray matter (PAG) in the rat has a high concentration of opioid analgesics and it is well established that injection of small amounts of morphine into this region induces analgesia. We have been studying the effects of antibodies to ganglioside on synaptically evoked responses in the rat midbrain (e.g., weak, moderate, strong) and unpleasantness (e.g., unpleasant, moderate, strong) of the sensory intensity (Sessle et al., Can. J. Physiol. Pharmacol. 54: 107-118, 1976), we tested for a PAD contribution to the depression. We found that all of 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 PAD produced by N. oralis and/or oralis is subject to primary afferent depolarization from PAG, NRM, VPM, and facial-skin sites; the ineffective nature of naloxone to reverse the PAD effect from N. caudalis and/or oralis that presynaptic opiate receptors might not be involved in the PAD.

(Supported by the Canadian MRC and NIH grant F1-ROL-DE04786-01.)


The region of the periaqueductal gray matter (PAG) in the rat has a high concentration of opioid analgesics and it is well established that injection of small amounts of morphine into this region induces analgesia. We have been studying the effects of antibodies to ganglioside on synaptically evoked responses in the rat midbrain (e.g., weak, moderate, strong) and unpleasantness (e.g., unpleasant, moderate, strong) of the sensory intensity (Sessle et al., Can. J. Physiol. Pharmacol. 54: 107-118, 1976), we tested for a PAD contribution to the depression. We found that all of 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 PAD produced by N. oralis and/or oralis is subject to primary afferent depolarization from PAG, NRM, VPM, and facial-skin sites; the ineffective nature of naloxone to reverse the PAD effect from N. caudalis and/or oralis that presynaptic opiate receptors might not be involved in the PAD.

(Supported by the Canadian MRC and NIH grant F1-ROL-DE04786-01.)

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 applied to active nociceptors. An easily controlled noxious stimulus is heat. STT cells were identified by antidromic activation from the centripetal termination in anesthetized monkeys (Macaca fascicularis). A thermal stimulator having a surface area of 14 cm² was placed against the glabrous 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, 67, and 50°C (rate 2°F/s). Stimulus duration was either 30 or 120 s with a 5 min inter-stimulus 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 temperature-modality sensitization to mechanical and to intense cold stimuli.

It is concluded that the responses of STT neurons in the primates 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.).


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 diencephalic 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 capacities of baclofen and morphine when microinjected at sites in the rat brainstem: the nucleus raphe magnus (RM), the brain stem nuclei shown previously to be active areas for morphine analgesia. On separate occasions, equiluminal doses of both agents (1.5 µg baclofen, 2.5 µg morphine sulphate) were microinjected 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 > 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 at only 3 of 16 PAG sites rostrally. Similarly, 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 the response to baclofen applied at rostral sites (AI = 0.29 ± 0.03). 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 = 0.16).

Baclofen was more effective 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 = 0.22) of 11 RM sites.

Despite the greater presurgical effect of baclofen 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.

1467 ANALGESIA ELICITED BY INTRACEREBRAL MICROINJECTIONS OF BACLOFEN AND MORPHINE IN RATS WITH CHRONIC PAINT. Jeffrey M. Liebman and Gary Pasto*. Research Dept., Pharmaceutical Div., Ciba-Geigy Corp., Summit, NJ 07901. Baclofen (B) and morphine (M) are two analgesic drugs which have been shown either to be active at different sites in the brain or to be different substrates involved in the analgesia produced by these agents. The present study was aimed at determining the effect of microinjection of B or M into various brain sites on the sensitivity to pain and on the capacity of both agents to produce analgesia in rats with chronic incisional pain. Male Wistar rats were stereotaxically implanted in dorsal tegmentum with guide cannulae (0.29 mm diam.), allowing for microinjection of 1 µmol 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 ventralateral mesencephalal central mesencephalal central gray, while BF and MUS analgesia was spread over a much larger area. The effects of BF were more potent than those of MUS. Although ataxia was also noted after some intracerebral microinjections of BF or MUS, it was not sufficiently severe to impair performance. BF was more potent intracerebrally than BF, but further investigations which employed the intraperitoneal route in rats showed, at best, weaker analgesic effects. BF was a GABA-mimetic, 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 GABAergic receptors, as by intracerebral microinjection of B or MUS, may provide a more specific antinociceptive effect. 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.

ANALGESIA ELICITED BY INTRACEREBRAL MICROINJECTIONS OF BACLOFEN AND MORPHINE IN RATS WITH CHRONIC PAINT. Jeffrey M. Liebman and Gary Pasto*. Research Dept., Pharmaceutical Div., Ciba-Geigy Corp., Summit, NJ 07901. Baclofen (B) and morphine (M) are two analgesic drugs which have been shown either to be active at different sites in the brain or to be different substrates involved in the analgesia produced by these agents. The present study was aimed at determining the effect of microinjection of B or M into various brain sites on the sensitivity to pain and on the capacity of both agents to produce analgesia in rats with chronic incisional pain. Male Wistar rats were stereotaxically implanted in dorsal tegmentum with guide cannulae (0.29 mm diam.), allowing for microinjection of 1 µmol 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 ventralateral mesencephalal central mesencephalal central gray, while BF and MUS analgesia was spread over a much larger area. The effects of BF were more potent than those of MUS. Although ataxia was also noted after some intracerebral microinjections of BF or MUS, it was not sufficiently severe to impair performance. BF was more potent intracerebrally than BF, but further investigations which employed the intraperitoneal route in rats showed, at best, weaker analgesic effects. BF was a GABA-mimetic, 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 GABAergic receptors, as by intracerebral microinjection of B or MUS, may provide a more specific antinociceptive effect. 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.

In previous work, we have used a signal detection theory (SDT) model to assess pain sensitivity and response bias in monkeys responding to trains of noxious electrical or mechanical stimuli. In the present study, we have recorded transcutaneous evoked potentials while subjects were performing in the SDT escape task. In order to obtain the best evoked potentials, subjects were exposed to 20 mg/kg/h sodium pentobarbital (2 mg/kg/h).  The spinal cord was exposed by partial cerebellectomy. A freed segment of sural nerve was electrodes introduced into the dorsal root entry zone at S1.  Firing indices were set to an intermediate value by adjustment of the stimulus strength.

The results indicate that there are at least two pathways for the transmission of nociceptive impulses to spinal neurons from afferent fibers carrying information from cutaneous and nociceptive afferents.  Studying the firing properties of (2, 200 µA. The latency of the PAD evoked by mechanical stimulation in these brainstem nuclei varied between 20 and 250 ms.  Stimulating in the NRM produced PAD in 20 A


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 in patients with pain, potential's were analyzed by averaging 1000 sweeps of the electroencephalogram by a Computer of Averaged Transients. Acupuncture was performed by using needle filaments in the Hoku point of the right hand. Naloxone was administered after acupuncture treatment. In 75% of our subjects the amplitude of the positive peak occurring at 28 msec (P28) decreased and the amplitudes of the late positive peaks, P46, and P53, decreased in 81 and 80% of our subjects respectively. We observed a concomitant decrease in latency to the P28 wave after acupuncture treatment. Moreover, the early negative peak (N18) amplitude decreased with the latency to and the amplitude of the late negative wave (N53) decreasing after acupuncture. Furthermore, in studies where naloxone was administered, there appeared to be a noticeable reduction in the amplitude of the late negative wave (N53) following naloxone alone. In our subjects there was a noticeable reduction in the amplitude of the P28 wave in the early stages of acupuncture treatment after acupuncture. The early components of the SEP represent conduction of nerve impulses through the spinal cord, and the late component are related well to behavioral response latencies, with the first group consisting of the 20 to 25 msec latency, followed by the second group of 100 to 150 msec latency. Thus, the behavioral intensity rating and not stimulus intensity was best correlated with the amplitude of these latter components. For the stimulus intensity of the late wave (3180 µV), the reverse was true. Potentials recorded from other cortical areas showed little correlation with behavior. The early components of the SEPs correlate with behavioral responses of magnitude less than 100 µV, and determination of the peak amplitude of the early component was made using the peak latencies of the late components.

In the present study, CSF was removed from persons with lower back and/or leg pain while they were undergoing spinal anesthesia for pain relief at the U.T. Pain Clinic. The CSF was then fractionated by a Computer of Averaged Transients. Acupuncture was performed by placing needles in the Hoku point and other relevant sites for the pain. 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: 259-266, 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 test the column fractions for endorphin activity with an opiate receptor binding assay (similar to: Brain Res. 88: 295-308, 1975). This assay confirmed most of the endorphin peaks indicated by the bioassay. Since there has been no apparent evidence for endorphin CSF in patients with pain, the level of endorphin was lower than that in patients with pain. Furthermore, the fact that we observe changes in the late events of the SEP, consistent with subject's reports of pain relief indicates that acupuncture produces changes in the supratentorial response to stimulation.


Stimulation in either the nucleus raphe magnus (NRM) or the nucleus reticularis gigantocellularis (NGc) in monkeys (Macaca fascicularis) results in the inhibition of spinthalamic tract neurons. Both ascending activity to the thalamus 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 excitatory and inhibitory afferent tests were recorded. In order to determine if primary afferent depolarization (PAD) results from stimulation in these brainstem nuclei, we observed changes in the firing activity of cutaneous and nociceptive afferents.  The firing rates were maintained with either a-chloralose or a combination of this agent and an infusion of sodium pentobarbital (2 mg/kg/h). The spinal cord was exposed by partial cerebellectomy. A freed segment of sural nerve was placed under a second freed segment. Glass microelectrodes were filled with 35% NaCl were used to record from different areas. 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 determined by stimulating the skin at the border between an area of no response and an area of a response. A persistent depolarization of the nerve was elicited by stimulation of the skin at the border between an area of no response and an area of a response.  The persistent depolarization of the nerve was elicited by stimulation of the skin at the border between an area of no response and an area of a response.  The persistent depolarization of the nerve was elicited by stimulation of the skin at the border between an area of no response and an area of a response.

In the present study, CSF was removed from persons with lower back and/or leg pain while they were undergoing spinal anesthesia for pain relief at the U.T. Pain Clinic. The CSF was then fractionated by a Computer of Averaged Transients. Acupuncture was performed by placing needles in the Hoku point and other relevant sites for the pain. 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: 259-266, 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 test the column fractions for endorphin activity with an opiate receptor binding assay (similar to: Brain Res. 88: 295-308, 1975). This assay confirmed most of the endorphin peaks indicated by the bioassay. Since there has been no apparent evidence for endorphin CSF in patients with pain, the level of endorphin was lower than that in patients with pain. Furthermore, the fact that we observe changes in the late events of the SEP, consistent with subject's reports of pain relief indicates that acupuncture produces changes in the supratentorial response to stimulation.


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 acupuncture (Psychopharmac. 18: 363-366, 1978). Furthermore, the fact that we observe changes in the late events of the SEP, consistent with subject's reports of pain relief indicates that acupuncture produces changes in the supratentorial response to stimulation.

(Supported by Anesthesiology Research Fund, Mt. Sinai Hospital)
STRESS-INDUCED ANALGESIA: CROSS-TOLERANCE STUDIES OF STRESSORS

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 local anesthetics in the PAG and intravenous morphine produces naloxone reversible behavioral hypalgesia. Similar changes in thalamocortical excitability are reflected in the intracortical microstimulation and PAG stimulation. We studied the effect of microinjection of morphine in the PAG to determine whether the PAG opiate receptors participate in the 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 the 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 the PAG all produced similar PI dependent alterations in ERP. These observations suggest that opiate receptors in the 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.)

STRESS-INDUCED ANALGESIA: CROSS-TOLERANCE STUDIES OF STRESSORS AND MORPHINE


Rats display elevations in pain thresholds when initially exposed to such stressors as a forced cold-water swim (CSW), 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 produces tolerance. We have previously found that cross-tolerance fails to develop between CSW-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 CSW 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 CSW injections (350 μg/kg) followed on the fifteenth, or cross-treatment day by morphine (10 μg/kg) in one group and CSW (2°C for 3.5 min) in the other. The third and fourth groups underwent 14 daily CSW or morphine injections, respectively followed by 2-DG injections on the fifteenth day. The fifth group underwent 14 daily warm-water (28°C) control shocks followed by 2-DG administration, the sixth group received 14 placebo injections followed by CSW. Acute exposure to 2-DG, CSW and morphine all produced analgesia; control shocks and injections did not. Repeated exposures resulting in the same conditions resulted in adaptation or tolerance. Full development of 2-way cross-tolerance occurred between CSW and 2-DG, in other word Groups 5 and 3 were equally effective in blocking PAG and intravenous morphine produced naloxone reversible behavioral hypalgesia. Similar changes in thalamocortical excitability are reflected in the intracortical microstimulation and PAG stimulation. We studied the effect of microinjection of morphine in the PAG to determine whether the PAG opiate receptors participate in the hypalgesic thalamocortical modulation.

(Supported by NIH Grant NS 14449 and N.Y.S. Health Research Council Grants #365 and #922.)

OPTIMUM DUTY CYCLE AND FREQUENCY OF INTERMITTENT ELECTROANALGESIA CURRENT


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 current (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 100 μsec pulses at 1000 μA have produces similar results, but duty cycles as low as 10% are equally effective in blocking pulp afferent activity (Fields et al. Exp. Neurol. 53:386-398, 1978). The present experiments were undertaken to examine the effect of duty cycle and frequency of pulsed electroanalgesia (EA) current that might produce blockade as effective as DC. We examined this study by 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, and 10,000 and 100,000 pps were subsequently exposed to 2-DG, but only partial (50%) cross-tolerance also occurred in chronic morphine-treated rats and #922.)

(Supported by NIH Grant DE 04281)

PAIN


Department of Neurology and Clinical Neuroscience Research Center, University of Virginia School of Medicine, Charlottesville, Va.

1474 ELECTROPHYSIOLOGIC ANALYSIS OF SYSTEMS SUBSERVING BASAL GANGLIA INHIBITION OF INTRALAMINAL THALAMIC NEURONS. Howard K. Strahler* and Charles D. Barnes. Department of Physiology, Texas Tech University School of Medicine, Lubbock, TX 79409.

Intralaminar thalamic nuclei are thought to play an important role in the extinction of affective pain systems. We have conducted a series of experiments in order to physiologically characterize the neural substrates of CN and SN inhibition on CN-Pf units in CM-Pf which responded in a characteristically 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 Forel's GP, thalamic reticular nucleus (RN), globus pallidus (GP), endopudicular nucleus (EN), locus coeruleus (LC), periaqueductal grey (PAG), and returns driven by either PAG or CN failed to display analgesia when switched to the other. Full cross-tolerance occurred between CWS and 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 CSW. (Supported by NIH Grant NS 14449 and N.Y.S. Health Research Council Grants #365 and #922.)


The present examination 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 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 administration is achieved utilizing the model described by Melzack (Pain 1: 277-99 1975).

Electrode configurations are also compared by evaluation of data recorded when placing electrodes ipsilateral or contralateral to the test tooth. Bilateral active electrode configurations are included. The data from all configurations will be presented and indicate that EA currents applied to both the buccal oral mucosa and to the mental foramina 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 foramina 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 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 for chronic pain in the head and neck including trigeminal neuralgia, temporal mandibular joint pain, migraine headache, maxillary and mandibular pain following trauma or surgical intervention. Evaluation of the effectiveness of EA current administration is achieved utilizing the model described by Melzack (Pain 1: 277-99 1975).


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; catatonic catatonia; lateral amygdala; cortico-medial amygdala; hippocampus; and the lateral thalamus. The flinch-jump technique was used to assess pain sensitivity and the bar test to study catatonia. Etorphine was administered in a single 0.1 ug or 0.01 ug dose. Etorphine was used only in one experiment and was administered etorphine only one time. The animals received a baseline bar test following a flinch-jump test followed by a second bar test (taking 10 min). Thereafter, the animals were injected with either etorphine or saline in a 1:1 solution into one neuroanatomical location. All animals received both water and etorphine, presented in a counterbalanced order and separated by a 10 minute interval.

Neuroanatomical location ranged from AP +3.0 to AP - 9.0, the region showing maximum sensitivity to the intracerebral administration of etorphine. Etorphine current, the nociceptive threshold is within the periaqueductal gray (N=16), a dose of 1 ug etorphine elicited significant analgesia and catatonia, while 2 ug etorphine elicited progressively stronger effects. The area showing the least responsiveness to etorphine-elicted analgesia or catatonia was the cerebellum (N=8). Neither 1 ug nor 2 ug doses elicited analgesia or catatonia. Injection sites were maxillary tissue. While 1 ug etorphine had no effect when injected into either the basolateral amygdala (N=8) or cortical-medial amygdala (N=2), 2 ug etorphine elicited significant analgesia and catatonia. A dose of 1 ug etorphine injected into the lateral amygdala elicited a significant increase in the latency to respond to a light tap on the head. The lateral amygdala and the dorsolateral columns, in various combinations, were made at 50 pm and reacted with O-dianisidine or tetramethylbenzidine. A total of 4439 cells (average of 555 cells per animal) were identified. There are also cells in the ventral part of lamina II which are labeled when HRP is injected into the lateral amygdala, the periaqueductal gray, the amygdala and the hypothalamus. There are also cells in the ventral part of lamina II which do not produce reliable analgesia or catatonia. All results are significant at the .01 level. Interesting results due to differences in needle placement will be discussed.

This study suggests that etorphine-elicted analgesia and catatonia seen after injection into the brain is site specific, is related to affinity of the site to binding sites for etorphine. The results do not just result from the diffusion of injection of etorphine into the site, but rather from the interaction between the site and the etorphine. This interaction may be a common mechanism and substrate for these two actions of the narcotic system.
PLASTICITY
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 brain in the adrenal medulla. The present studies were designed to investigate whether a similar increase in TH activity might occur in residual noradrenaline (NE)-containing nerve terminals in brain following intraventricular injection of 6-HDA. Rats were given a single intraventricular injection of 6-HDA (200 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 ^4CO_2 from carboxyl-labeled [14C]-L-tyrosine (75 µM) in the presence of an excess of DOPA decarboxylase and saturating 6MPA. 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 63% 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 day 12. Time courses for the appearance of increased TH activity have been reported following reserpine. Further experiments will determine whether the increased TH activity is due to an increased synthesis 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 MH00038 and MH02620.}


Serotonin release by blood platelets is a process similar in many respects to neurotransmission 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 (pl=9.1), a 20K dalton protein phosphorylated in a manner which correlates with the time course of and extent of release. Additionally a 40K dalton protein (pl=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 protein phosphorylation is necessary, but not sufficient in itself, for release. The 40K basic protein has tentatively been identified as tropomin-T, and in view of the known role of tropomin in regulation of calcium sensitivity in muscle contraction, we propose that the phosphorylation of tropomin-T confers calcium sensitivity upon the process of serotonin secretion.


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 to correspond to 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 reared 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/deg) in one cat and normal in the other (4.5 c/deg). Reconstruction of the retinal lesions from fundus photographs and whole mounts of the retina showed that in both cases the lesions were confined 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. Acquisitions of novel 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 of cats reared under these conditions was able to perform 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 MD cats are being visioned 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 09913, 01565 and NSF BMS 7513877, 7707885.
NIH Grant #2RO1-NS 12330-02A1 to AJB.

Before surgery, eye movements to the pulsed bridges may become root afferents may mediate the behavior observed. (Supported by

Central representations of relevant movements may be established and retrained by covering the response panel, except for the start button. This required 10-12 trials without error with randomly presented single or dual sequent flashes. If the monkey failed to touch the bridge within 3 seconds 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 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 usually 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 (0.5/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 5 sec the trial was terminated.

Preoperatively, monkeys reached criterion (bridge touched 9/10 trials without error) with randomly presented single or dual bridges within 7-21 days after surgery, performance with the intact limb was unaffected. Movements of the rhizotomized limb, however, had to be shaped. This was accomplished by shaping the response by either exclusion of 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 in darkness as the bridges were introduced. Monkeys were successful in touching visually cued targets with their rhizotomized limbs. There was a shorter latency, however, the variability in session to session with the rhizotomized than the intact limb.

Several alternative explanations for these results are offered. Central representations may be established and retrained with different commands after surgery. Transfer of the intact limb may have facilitated performance. Finally, voluntary root afferents may mediate the behavior observed. (Supported by NIH Grant NS 280-1-25 12330-02A1 to AJB)


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 mosmol and pH 7.2 which included MgCl₂ (2 mM) and either CaCl₂ (8 mM) or BaCl₂ (0.8 to 8 mM). Cells were impaled with two intracellular glass 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 rise (dV/dt) or in the spike amplitude; if anything, both decline.

Bariun 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 mulluscan neurons, where it has been hypothesized (Thompson & Getting, Neurosci. Abstr. 3, 189, 1977) to result from cumulative inactivation at a TCA-sensitive voltage-dependent channel of an inward Ca current. Our results are consistent with this potassium inactivation hypothesis. (Supported by NIH Grant NS 12151).


The presynaptic stores of acetylcholine in the cat superior cervical ganglion can be produced by 30-70% (40-70 Hz) presynaptic stimulation with patterns of pulses whose mean frequencies are within physiological limits (0.2-3/0.4). The increases in transmitter stores in response to presynaptic stimulation occur only after termination of the stimulation (Birske, R. J., Physiol. in Press, 1978). It has now been found that these increases 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 (Bubnick, R.O., 1976) to cause Ca⁻⁺ spike broadening and an increase in transmitter stores in the sympathetic nerve terminals. (Supported by NIH Grant NS 276. 331-320, 1978) to occur in animals under stress. The increase in stores was reduced or prevented by arterial infusion of nor-epinephrine or epinephrine at a rate of 0.05-1.0 mg/kg/min during patterned stimulation in animals that retained their spinal ganglionic circulation intact. Similar depression of the response to patterned stimulation was obtained in ganglia perfused with choline-locked when the solution contained nor-epinephrine or epinephrine at a concentration of 5 mM. Since the increase in transmitter stores has also been shown to be reflected in a corresponding increase in transmitter output (Birske, R. J. Physiol. 221, 847-867, 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.)


In the fish (Cichlasoma bicellatum), possessing a flattened, rhomboid-like ON, the observations confirm our previous work (Bunt, S. H., and Horder, T. J. J. Physiol. 210, 120-127, 1971). In the fish (Cichlasoma bicellatum) the retinal ganglion cells are arranged in columns separated by inhibitory processes. We found that the injection of tracer into the optic nerve (ON) results in a line of degeneration along the course of the optic nerve. The injection was made at the beginning of an elongated segment of the optic nerve, and tract and bundle elements were followed by light microscopy of 200-400 µm sections. The topographical arrangement of optic fibers within the retina 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.

As part of a study of the effect of chronic immobilization on medial gastrocnemius (MG) motor unit properties, we examined the peak amplitude of composite homogeneous (MG) and heterogeneous (lateral gastrocnemius - soleus, or LGS) group Ia EPSPs in motoneurons of dependent motor units (Burke et al., J. Neurophysiol., 33:723, 1970). 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 and for changes in the commissural-associational (CA) fiber plexus as in a previous study of Ia EPSPs in normal cats (Burke et al., J. Neurophysiol., 49:1976). Table 1 shows the comparison between Ia EPSPs in normal and immobilized animals.

### Table 1

<table>
<thead>
<tr>
<th>Unit Type</th>
<th>Source</th>
<th>Term (N=2)</th>
<th>Term (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>MG</td>
<td>4.1 ± 1.5</td>
<td>2.8 ± 1.3 (14)*</td>
</tr>
<tr>
<td>LGS</td>
<td></td>
<td>1.2 ± 0.6</td>
<td>0.6 ± 0.1 (15)*</td>
</tr>
<tr>
<td>FR</td>
<td>MG</td>
<td>7.5 ± 1.9</td>
<td>4.2 ± 2.4 (7)*</td>
</tr>
<tr>
<td></td>
<td>LGS</td>
<td>2.0 ± 0.9</td>
<td>1.2 ± 0.3 (7)</td>
</tr>
<tr>
<td>S</td>
<td>MG</td>
<td>9.1 ± 1.8</td>
<td>6.2 ± 1.5 (3)</td>
</tr>
<tr>
<td></td>
<td>LGS</td>
<td>2.8 ± 1.1</td>
<td>1.2 ± 0.5 (4)*</td>
</tr>
</tbody>
</table>

* = 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 Ll) of defined motor unit type (Burke et al., J. Physiol. 234, 723, 1973). Unit EPSP Normal (N = 10) Short Long

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 chronic administration of corticosteroids results in both cortical atrophy and new synapse formation following partial denervation. The hippocampus has also been shown to have specific receptors for corticosteroids, one of which appears to be localized in the dentate gyrus. Possibly 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.


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 chronic administration of corticosteroids results in both cortical atrophy and new synapse formation following partial denervation. The hippocampus has also been shown to have specific receptors for corticosteroids, one of which appears to be localized in the dentate gyrus. Possibly 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 pre-operatively 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 commissuronal-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 occurred. 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 occurred. 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 occurred. 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 occurred.

These findings raise the question of whether corticosteroids administered to prevent edema decrease rather than enhance the rate and extent of 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 corticosteroids exhibit anti-inflammatory and hypertrophy of astrocytes. Thus altered hormonal levels may possibly be one of the factors responsible for reduced axon sprouting following cell loss in aged animals. (Supported by research grants AG 00538 and NS 05897)

In 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 Neurological Behavior Biology Prog., Univ. IL at Urbana-Champaign, IL 61802.

The amount of branching of dendrites is affected by the rearing environment; in the present study, the number of dendritic segments was small but consistent differences in dendritic branching between exclusively maze-trained adult rats and handled rats. These differences appeared in all types of dendrites and were statistically significant. The results suggested that contact eye occluder system to direct visual aspects of the training experience to a single hemisphere. Six groups of 60-70 day male littermates were examined. The control group consisted of the dorsal dience of layer 5 pyramidal cells of occipital cortex.

To study general stimulation versus specific aspects of the maze training experience, we used a specific target paradigm. The rat was trained to make a contact eye occluder system to direct visual aspects of the training experience to a single hemisphere. Six groups of 60-70 day male littermates were examined. The control group consisted of the dorsal dience of layer 5 pyramidal cells of occipital cortex. The results suggested that contact eye occluder system to direct visual aspects of the training experience to a single hemisphere. Six groups of 60-70 day male littermates were examined. The control group consisted of the dorsal dience of layer 5 pyramidal cells of occipital cortex.

**EXPERIMENTAL 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 Neurological Behavior Biology Prog., Univ. IL at Urbana-Champaign, IL 61802.

The amount of branching of dendrites is affected by the rearing environment; in the present study, the number of dendritic segments was small but consistent differences in dendritic branching between exclusively maze-trained adult rats and handled rats. These differences appeared in all types of dendrites and were statistically significant. The results suggested that contact eye occluder system to direct visual aspects of the training experience to a single hemisphere. Six groups of 60-70 day male littermates were examined. The control group consisted of the dorsal dience of layer 5 pyramidal cells of occipital cortex. The results suggested that contact eye occluder system to direct visual aspects of the training experience to a single hemisphere. Six groups of 60-70 day male littermates were examined. The control group consisted of the dorsal dience of layer 5 pyramidal cells of occipital cortex.


Anastomoses exist between branches of the three major cerebral arteries in humans and animals. 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 in the vascular organization of the affected hemisphere.

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 frontal and zygomatic vessel foundations was resected. Anastomoses exist between branches of the three major cerebral arteries in humans and animals. 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 in the vascular organization of the affected hemisphere.

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 frontal and zygomatic vessel foundations was resected. Anastomoses exist between branches of the three major cerebral arteries in humans and animals. 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 in the vascular organization of the affected hemisphere.
The rostromedial subdivision of the lateral posterior nucleus (LP) in the golden hamster is normally characterized by dendritic appendages of a single dendrite. These terminals occasionally extend into the synaptic clusters but they are easily distinguished 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. 2:295, 1970) and the remaining colliculus contribute many more terminals to LP than usual. By removing the contralateral colliculus and injecting the contralateral eye with 3H-leucine and then processing adjacent sections for autoradiograms and autoradiographs, 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 rostromedial 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 the contralateral eye compete with complex dendritic appendages of a single dendrite. These terminals form many synaptic terminals but continue to compete with each other for postsynaptic space. Supported by NIMH Grant NS-09623 to W. C. Hall.
ASYMMETRICAL DENDRITIC DEVELOPMENT OF NEURONS IN THE ACCESSORY SUPERIOR OLIVARY NUCLEUS OF ALBINO RATS RAISED UNDER MONOURAL "DEPRIVATION".* Albert S. Fung. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Neurons in the mammalian accessory superior olivary nucleus receive inputs from the ipsilateral cochlear nucleus and the contralateral cochlear nucleus. After the contralateral cochlear nucleus is removed laterally from the ipsilateral cochlear nucleus (Stotler, 1953; Liu and Liu, 1971), these neurons serve as an excellent model system in the precise interconnection 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 decreased numbers of synaptic contacts. The deprived 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, were 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.
AGE-DEPENDENT REACTIONS OF OLFACTOR Y CORTEX TO DEAFFERENTATION.

The reaction of piriform cortex to deafferentation has been studied in rats using the Timm stain (Haug, 1973) and the autoradiographic method (Haug et al., 1974) following a bilateral ablation of the olfactory bulb in developing and mature rats. The Timm method stains heavy metals in axons, and in normal animals it reveals the pattern of termination of axons in piriform cortex. The superficial lamina (1a) is very pale while the deep lamina (1b) shows a dark rust colored reaction. In addition, layer 1 extends to a lesser extent in a granular reaction which is most prominent in the outer edge of 1b. The pale and colored laminae correspond precisely to the layers of termination of axons from the olfactory bulb (in 1a) and of olfactory association fibers from the olfactory cortex (in 1b) (Price, 1973), but the granular reaction does not correspond to any known extrinsic fiber system. Some indication of a bilaminar Timm reaction in layer 1 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 on P-1 (2-12 weeks survival) layer 1 develops to its normal thickness but the granular and rust colored reactions are found throughout this layer. This indicates that the association fibers extend to the superficial part of layer 1, demonstrated in parallel autoradiographic experiments.

In contrast, in adult rats (2-8 months survival) layer 1 is reduced in width by about 25%, probably due to dendritic degeneration. The normal color reaction in 1b is preserved here. There is a preferential loss of granular reaction in 1a which becomes much more intense with longer survivals. The association system, demonstrated autoradiographically, is largely confined to 1b, in correspondence to the color reaction.

Following ablations at ages 4-14 days (2 month survival) there is a reduction of layer 1 to the width of the adult (1b) which is almost comparable to that seen after ablations made in adult animals. However, the staining pattern is similar to that seen in P-1 ablations; the density of the rusty colored reaction is fairly homogeneous throughout layer 1 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 1 but are concentrated in its deeper part. Supported by NIH Grants NS09518 and NS07057.

ANOMALOUS CROSSED CORTICOCORPOUS PROJECTIONS IN THE POSTNATAL MONKEY INDUCED BY PERNAL SECTION OF FRONTAL NEOCORTEX.
Patricia R. Goldberg and M.C. Landa. Dept. Physiol., Univ. of Md., Baltimore, Maryland 21201.

Primates, including man, exhibit remarkable sparing of behavioral performance following circumcortical brain injury that occurs early in life. It is not known, however, to what degree primates exhibit a capability to respond to such injury by reorganization of axonal connections. To examine this issue, the prospective development of prefrontal cortical projections to the hemisphere of a fetal rhesus monkey 6 weeks prior to birth. Following surgery, the fetus was returned to the uterus and subsequently reared in the usual manner. At birth, the homotopic region of the dorsolateral cortex contralateral to the cortex that was resected prenatally was injected with microquantities of H253. Autoradiograms were traced in consecutive serial sections throughout the entire extent of layer I of the homotopic region. The autoradiograms were compared with those from animals of the same age in which no resection was performed. The autoradiograms from normal 5-day old monkeys that had been injected with microquantities of H253 were examined. The autoradiograms were compared with those from animals of the same age in which no resection was performed. The autoradiograms from normal 5-day old monkeys that had been injected with microquantities of H253 were compared with those from animals of the same age in which no resection was performed. The autoradiograms from normal 5-day old monkeys that had been injected with microquantities of H253 were compared with those from animals of the same age in which no resection was performed. The autoradiograms from normal 5-day old monkeys that had been injected with microquantities of H253 were compared with those from animals of the same age in which no resection was performed. The autoradiograms from normal 5-day old monkeys that had been injected with microquantities of H253 were compared with those from animals of the same age in which no resection was performed.

Several forms of potential plasticity at the synaptic level have been described following exposure to differential rearing conditions. In this study of differential rearing upon experience synapses, we have examined interruptions in the darkly staining region underlying the post-synaptic membrane. Peters and Kalsenman-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 47,800x. 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 3 and 4 (as determined from adjacent light microscopic sections). Across layers, complexity-reared rats had 255 more synapses with SSPP's (p<0.05) than the isolates in the SSPP's synapses examined. Socially-reared rats were intermediate. While percentages differed, the effect was seen in all 3 layers.

A second experiment in which male littermates housed either in pairs or in isolation for 130 days following weaning. The socially-reared rats' synapses had a 45% higher frequency of occurrence of SSPP's than those from isolates (p<.05). The frequency of SSPP's has shown to be higher in rats that have experienced an intensive 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 increased at a slower rate 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 changing relationship of post-synaptic anatomy in response to experience which could underlie functional changes in synaptic efficacy or permanence.

Supported by Grants HD 08682, NSF BMS 75-08596, and NSF BNS 77-23660.


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 of distal dendritic and mostly in the spinocellular region of the nucleus with a few found caudally. Cerebellorubral endings were found on somata, dendritic shafts and on distal reticulocellular and caudal thalamic terminals of the nuclei 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 1-3 days the lesions were scrutinized and found to be intact. Following bilateral removal of the 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 controls. The remaining 4 were sacrificed at 3, 7, 14, and 21 days. The results showed that with the rostral and caudal lesions at 37 days, there were no surviving collaterals. However, in the remaining cats, there were many surviving collaterals in the red nucleus.


Unilateral stimulation of the entorhinal cortex (EC) leads to the gradual development of seizures in response to the stimulation (kindling). We have found that the rate of kindling observed in animals with no lesions, is slower than those with bilateral EC stimulation, and after 3 days animals were sacrificed and perfused with 3L of 1% paraformaldehyde and 1.5% gluteraldehyde. The red nucleus was identified on 250 unsectioned blocks which were cut anteroposteriorly and obtained from the dorsal and lateral aspects of the EC. The procedure for these results was to examine whether the enhanced transfer can take place before 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 in the contralateral EC2 were examined, 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 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 altered, and given a total of 6.1 trials, a rate significantly slower than that for kindling following destruction of EC1; 2) by omitting the initial kindling the kindling remained when transynaptic changes would increase the rate of kindling. This group required an average of 35.5 trials for kindling, which was not significantly faster than the initial kindling in this group. 3) by beginning EC2 kindling on day rather than day post lesion, we examined whether the enhanced transfer can take place before the kindling has occurred. The group in the second group did not increase the rate of EC2 kindling and after 10.3 trials in this group, which was not significantly faster than the rate of second kindling observed in animals with no lesion. These results are consistent with the hypothesis that the kindling of the EC2 is due to secondary kindling from the primary kindling which has been transsynaptically altered by the kindling stimulation.

(Supported by NIH Grant NS12393. MEH is a postdoctoral trainee (NS 7044)).

The cytoarchitectonic appearance and of the pattern of thalamic afferents to the barrels of the somatosensory 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 sixteenth day of life (PND-16) results in 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 neural losses in row C.

To study the effects of vibrissae damage on the morphology of individual barrel neurons, the vibrissae row C of mice was cataractized on PND-1, 1.5, or 4. The animals were sacrificed on PSD-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 specimens are comparable to those previously described. Typically, the vibrissae row C is totally undamaged, showing that retinotectal fibers can project to the postnatal age at the time of the lesion; (c) damage to one of the parietal cortex 76%(5) 52%(6)* 467.(4)* 6 77.(2)

Frontal Cortex 85%(5) 40%(6)* 35%(6)* 357.(5)*

Hippocampus 86%(5) 47%(6)* 36%(6)* 367.(5)*

Cerebellum 55%(4)* 30%(6)* 18%(5)* 223.(2)*

Hypothalamus 67%(5) 25%(6) 71%(5) 47%(2)*

*Percentage control value. Number of samples in parenthesis.


The following three surgical procedures were performed on eight newborn hamsters: a) bilateral right eye enucleation; b) 1.5-mm deep slit made through the skull along the midline between the pons and medulla (p-m) in animals of the following age: 1, 2, or weeks† 4 weeks† normal midline damage, 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 reticulotectal (TH) nerve terminal volume, which is proportional to TH activity in the pons, 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 was the same. Damaging the right SC at its terminal volume, the surface areal distribution of the fibers in the left SC increased and spread laterally in proportion to the damage done to the tectal SC. Correlations were also examined in 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 # EY0126, by Insurance Medical Scientist Scholarship Fund, Mass Mutual Life Insurance Co., and by Harvard-MIT Health Sciences and Technology Division.

1152 PROLONGED CHANGES IN BRAINSTEM TYROSINE HYDROXYLASE ACTIVITY FOLLOWING NEONATAL CEREBELLECTOMY. L. Iacovitti, T.H. Joh, D.J. 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 reticulotectal fibers (thus preventing the generation of a regenerative response), the effect of 6-hydroxydopamine (6-OHDA) on central noradrenergic cells has been less well understood. We wished to determine what the effects of injection of 6-OHDA into locus coeruleus (LC) itself would have on the morphology and caudalolateral levels of this homogeneous population of noradrenergic cells. We have therefore examined the effects of lesions of the LC on the noradrenergic (NE) neurons in selected areas of rat brain known to receive LC projections. We have also sought to determine whether damage to the LC produced a less than a normal area of the neuron by anoxia is also seen following lesions of the cell body. A stereotactic template was used to make a 2 µl solution of 6-OHDA into the IC 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, and 4 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)* 357.(5)*

Parietal Cortex 76%(5) 52%(6)* 46%(6)* 67(2)

Hippocampus 86%(5) 47%(6)* 36%(6)* 367.(5)*

Cerebellum 55%(4)* 30%(6)* 18%(5)* 223.(2)*

Hypothalamus 67%(5) 25%(6) 71%(5) 47%(2)*

*Percentage control value. Number of samples in parenthesis.

Supported by NIH grants NS-11650 to T.H. Williams from the N.I.H.
In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (coral zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all areas 17 and 18 and the first few weeks of life. In later life, the CZ is restricted to the region almost about the line of the line of the second month of postnatal life. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience during the 1st-2nd month of life can affect the development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) one in one eye, by eyelid suture maintained from postnatal day 7 (or prior to squinting) and then, at 7 days of age, 14 (or postnatal day 9) of age, 14 unexpected visual stimuli 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 these kittens was 2-5 deg in the normal. Radial location and morphology of callosal neurons are typical of the visual cortical receptive fields of the normal adult cat. In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (coral zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all areas 17 and 18 and the first few weeks of life. In later life, the CZ is restricted to the region almost about the line of the line of the second month of postnatal life. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience during the 1st-2nd month of life can affect the development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) one in one eye, by eyelid suture maintained from postnatal day 7 (or prior to squinting) and then, at 7 days of age, 14 (or postnatal day 9) of age, 14 unexpected visual stimuli 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 these kittens was 2-5 deg in the normal. Radial location and morphology of callosal neurons are typical of the visual cortical receptive fields of the normal adult cat. In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (coral zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all areas 17 and 18 and the first few weeks of life. In later life, the CZ is restricted to the region almost about the line of the line of the second month of postnatal life. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience during the 1st-2nd month of life can affect the development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) one in one eye, by eyelid suture maintained from postnatal day 7 (or prior to squinting) and then, at 7 days of age, 14 (or postnatal day 9) of age, 14 unexpected visual stimuli 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 these kittens was 2-5 deg in the normal. Radial location and morphology of callosal neurons are typical of the visual cortical receptive fields of the normal adult cat. In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (coral zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all areas 17 and 18 and the first few weeks of life. In later life, the CZ is restricted to the region almost about the line of the line of the second month of postnatal life. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience during the 1st-2nd month of life can affect the development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) one in one eye, by eyelid suture maintained from postnatal day 7 (or prior to squinting) and then, at 7 days of age, 14 (or postnatal day 9) of age, 14 unexpected visual stimuli 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 these kittens was 2-5 deg in the normal. Radial location and morphology of callosal neurons are typical of the visual cortical receptive fields of the normal adult cat. In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (coral zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all areas 17 and 18 and the first few weeks of life. In later life, the CZ is restricted to the region almost about the line of the line of the second month of postnatal life. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience during the 1st-2nd month of life can affect the development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) one in one eye, by eyelid suture maintained from postnatal day 7 (or prior to squinting) and then, at 7 days of age, 14 (or postnatal day 9) of age, 14 unexpected visual stimuli 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 these kittens was 2-5 deg in the normal. Radial location and morphology of callosal neurons are typical of the visual cortical receptive fields of the normal adult cat. In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (coral zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all areas 17 and 18 and the first few weeks of life. In later life, the CZ is restricted to the region almost about the line of the line of the second month of postnatal life. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience during the 1st-2nd month of life can affect the development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) one in one eye, by eyelid suture maintained from postnatal day 7 (or prior to squinting) and then, at 7 days of age, 14 (or postnatal day 9) of age, 14 unexpected visual stimuli 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 these kittens was 2-5 deg in the normal. Radial location and morphology of callosal neurons are typical of the visual cortical receptive fields of the normal adult cat.
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 [3H] proline 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 thoro­

cric 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 was studied in 1 to 8 day old hamsters. Autoradiographs showed massive and abnormal decussation of the pyramidal tract several mm. rostral to the lesion. These aberrant fibers follow an abnor­
mal 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. The terminations of the lesions in the anterior horn of the cord. This new pathway develops in animals in which the pyra­midal tract had reached lumbar levels but had invaded the dorsal horn only as low as the mid-thoracic cord.

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 colli­

que (SC) is densest in the background at this time. Between 6 and 9 days postnatal, the normal uncrossed projection to dLGN becomes localized as much in adult animals and the projection to SC is more clearly focused anteriorly in the stratum opticum.

The uncrossed projection to SC in uncut animals is already distinct from controls by 2 days, with grain levels above back­
ground throughout the stratum griseum superficiale. However, the lateral geniculate projections 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 projections to dLGN cannot be distinguished as early as 4 days postnatal in animals 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 chiasma after emulculation. (Supported by USPHS Grants ET-05185 and ET-00596.)

Aided by USPHS Grant No. 25643 awarded to M.S. Gazzaniga.

When one or both habenulae are removed from a rat in the first days following birth, several changes in the synapticology 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 assayed this phenomenon quantitatively by preparing 10 normal and each-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 (x15,000), and each apparent synapse was photographed at high magnification (x30,000). The only selection made was an attempt to approximately equilibrate 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 photograph and counted. The perimeter of each perikaryon was measured. The results were expressed by several microscope observations of the 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 16.67, with a mean of 12.63. This 105% increase was significant as judged by a two-tailed Student t test (p<0.01). The endpoints from the synapses contained pleomorphic vesicles including at most one or two true flattening vesicles in almost all cases. Occasion and occasional 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 endpoints with prominent flattened vesicles.

It is concluded that the qualitative observation was correct although the magnitude of the increase was greater 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 NS 00169.

SHORT-TERM SYNAPTIC MODULATION IN THE MEDIAL AND LATERAL COMPONENTS OF THE PERFORANT PATHWAY. Bruce I. McNaughton, Dept. of Psychology, Dalhousie University, Halifax, N.S. CANADA B3H 4J1.

As indicated by their discrete pattern of heavy metal accumulation (e.g., Limmer & I. North-Simonsen, J.Comp.Neuro. 1975, 161, 7-17), 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.Neuro. 1976, 167, 255-314) 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.Neuro. 1977, 175, 439-454) that the extracellularly recorded synaptic responses of these two components could be distinguished 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 pento-barbital 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.
1525 

In previous experiments we have shown that transaction of the spinal cord at T13 or L5 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 technical procedures we have recorded EPSPs in homonymous motoneurons immediately following spinal cord transection at either T13 or L5. Following transaction uncommonly large EPSPs (> 400 µV) were observed but only after several hours had elapsed. These data in intact anesthetized preparations. In the present experiments done using identical technical procedures we have recorded EPSPs in virtually all homonymous motoneurons in contrast to several MG motoneurons prior to spinal cord transection at either T13 or L5. Following transaction 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. Propranolol block followed by transaction at that level resulted in no change in EPSPs in cells recorded continuously throughout this procedure. We conclude that enhancement 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 fast motoneurons. Their rise times and half widths were clustered near the origin of the shape index curve (half width Vic. rise time), suggesting a selective enlargement of EPSPs produced by IA terminals synapsing proximally on the motoneuron. Propranolol block followed by transaction at that level resulted in no change in EPSPs in cells recorded continuously throughout this procedure. We conclude that enhancement of EPSP amplitude is not an immediate consequence of loss of descending input.

Immediately following transection, single IA fibers evoked EPSPs in virtually all homonymous motoneurons in contrast to the projection frequency of 78% previously reported by Mendell and Mendell (J. Neurophysiol. 33: 679-692, 1970) in cats with intact spinal cords. No lower projecting type Y afferents (Collatos and Mendell, J. Physiol. Lond.) 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 increase in IA afferent activity of hitherto "silent" synapses. The link between cord transection and the enhancement of IA evoked individual EPSPs recorded in homonymous motoneurons is not yet known. (Supported by NIMH.)

1526 

A reversible reduction in the activity and amount of the neurotransmitter synthesizing enzymes, tyrosine hydroxylase (TH) and dopamine-β-hydroxylase, presumably a consequence of reduced neuron biosynthesis (Ross et al., Neuroscience Abstr. 4, 431, 1977) characterizes the response of central dopaminergic and noradrenergic neurons to the lactation reaction. We have measured the activity and amount of TH and dopamine-β-hydroxylase in hypothalamic neurons during lactation and in lactation-deprived rats, using a specific antibody to CAT prepared from rat caudate nucleus demonstrated the reduction in CAT activity was entirely due to reduced accumulation of CAT protein may reflect reordering of patterns of protein biosynthesis favoring production of proteins for reconstituting neurotransmitter biosynthetic pathways. This suggests that neuron biosynthesis is reduced during lactation by a mechanism that precedes the reduction in enzyme activity. The specific antibody to CAT prepared from rat caudate nucleus demonstrated the reduction in CAT activity was entirely due to reduced accumulation of CAT protein.

This apparent progressive loss is being investigated in further 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 experience did not protect against remote loss contrary to a hypothesis suggested in Will et al. (Science, 1978) ch. 33 characteristic of the response of central dopaminergic and noradrenergic neurons to the lactation reaction. We have measured the activity and amount of TH and dopamine-β-hydroxylase in hypothalamic neurons during lactation and in lactation-deprived rats, using a specific antibody to CAT prepared from rat caudate nucleus demonstrated the reduction in CAT activity was entirely due to reduced accumulation of CAT protein may reflect reordering of patterns of protein biosynthesis favoring production of proteins for reconstituting neurotransmitter biosynthetic pathways. This suggests that neuron biosynthesis is reduced during lactation by a mechanism that precedes the reduction in enzyme activity. The specific antibody to CAT prepared from rat caudate nucleus demonstrated the reduction in CAT activity was entirely due to reduced accumulation of CAT protein. 

This research was supported by grants from the Raiser Seal Foundation and ADDA Grant 801 M26704. It also received support from the Division of Biomedical and Environmental Research of the U.S. Department of Energy through the Lawrence Berkeley Laboratory.


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 hydroxydopamine (6-OHDA) treatment to dorsal brain and spinal cord (Cr. & Pet., Br. Res. 79, '74). Stimuli to nearby ventral regions (Cr. & Pet., Soc. Neurosci. Abstr. 1, '75) also modify the PDR (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 treated with 6-OHDA during the first 2 or 4 postpartum days (Day 0 = birth). The latency, duration and intensity of the response, however, were still significantly different from controls. Amphetamine (4 mg/kg) and 1-DOPA (100 mg/kg) were ineffective, while apomorphine (8 mg/kg) abolished the PDR in 6-OHDA treated pups; recovery was not evident at 90 days of age.


These data indicate that NE neurons of descending bulbospinal pathways are essential for the development and maintenance of the PDR, and that amphetamine and apomorphine may influence several aspects of this reflex.
THE DEVELOPMENT OF PLASTICITY IN THE HIPPOCAMPUS. Timothy J. Tyler and Charles Duffey*. 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 also reflect the plasticity exhibited by the developing brain. The normal development of neuronal response plasticity has, however, been neglected in this area of research. Our studies have suggested that the development of plastic processes was compared to the morphological development of dentate granule cells at each age, using rapid Golgi techniques.

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. The development of dendrites is described as: (1) The establishment of primary dendrites, (2) The formation of dendritic spines, (3) The elaboration of the dendritic arborization, and (4) The reorganization of spine density.

The results may indicate that the mechanisms underlying post-tetanic potentiation may be different from those underlying 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 synaptogenesis, spine formation, dendritic arborization and the reorganization of dendritic input pattern.


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.

The results of these experiments also indicate 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.)
Areal measurements of the size and counts of the number of vesicle after spinal cord injury if the damage occurs before synaptogenesis age, followed by a more moderate increase in sectional area after VII remained constant at a small value (≃0.37 µm²) until 12 days of comparable magnitude, in area of the gray matter until 15 days were performed on material from the same region in animals 3, 12, VII. This region was selected for study since it is an interneuron behavior recovery.

Prior to the twelfth postnatal day, spinal shock is minimal and region of the lumbar spinal cord proceeds rapidly between birth term and later in development little recovery occurs and the classical different behavioral results which depend upon a rat's age at the lateral portions of the intermediate gray matter, laminae VI and VII. This region was selected for study since it is an interneuron picture of paraplegia is observed. (Brain Research 125:241—255; 1979. Eric D. Weber* and Dennis J. Stelzner (SPON: James A. Horel).

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 22903.

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 sural nerve axons to the partially denervated DG (Steward, 1969). Among these changes is a proliferation of terminals of the normally sparse crossed temporodentate pathway originating in the contralateral entorhinal cortex (CCE). As part of an effort to ascertain the extent to which the 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 with long-standing IEC lesions, delivery of identical short, high frequency stimuli to the CCE elicited the synaptic response elicited via the sprouted crossed temporodentate connections. No evidence of potentiation of the contralateral IEC of the lesioned side was observed in the control group. Further, no change in the increased synaptic drive associated with LTP in the lesion-induced crossed temporodentate pathway did not, in contrast with LTP, indicate that the enhanced synaptic drive observed in the lesion-induced pathway is a result of increased synaptic drive. The results suggest that the lesion-induced and normal connections possess similar synaptic capacities. Since the enhanced synaptic drive is not accompanied by an enhancement of granule cell discharge, the results support the hypothesis that the lesion-induced synaptic drive is a result of increased synaptic drive. (Supported by U.S. Public Health Service Grant # RO1 NS13133 to O. Steward.)


Previous studies of the effects of environmental deprivation on mammalian development have been largely at the cellular and molecular level. By observing the effects of environmental deprivation on the late postnatal development of the Purkinje cell dendritic tree, we can gain insights into the functional consequences of altered early life experiences. Therefore, the effects of environmental deprivation on the late postnatal development of the Purkinje cell dendritic tree were investigated. Images of computer generated morphology, obtained from observations of C57Bl/6J hybrid mice were placed in either a deprived or enriched environment after weaning at 18 days of age and stored for 35 days of age. Depri ved animals were reared in a large cage with toys and a running wheel 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 in deprived animals of 9.6% in dentritic field areas and 9.2% in total dentritic 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 abovementioned effects were seen in various lobules of 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 an early stage in the growth of their dendrites. These results suggest that synaptogenesis in this region of the lumbar spinal cord proceeds rapidly between birth and 12 days of age but 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 NIH Grant #10657-D1.)

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL STABILITY OF THE MAUThNER CELL FOLLOWING AXOTOMY BY SPINAL CORD TRA NSECTION. Stavros I. Zottoli and Donald S. Faber. N.Y.S. Res. Inst. on Alcoholism, 1021 Main Street and Dept. Physiol., SUNY at Buffalo, N.Y. 14203, U.S.A.

In order to explore the potential of the Mauthner (M-cell) system for studies of neuronal plasticity, we have analyzed its behavioral, morphological and electrophysiological responses to axotomy performed by spinal cord or bulb transections at different levels of the spinal cord. 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 muscles in 171 trials conducted from 1-22 days postoperatively (n=15) 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. 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 iplateral VIIIth nerve stimulation and antidromically by stimulation of the cut spinal cord. Although the apparent lack of an axon reaction is due to a minimal trophic influence exerted upon the M-cell by the efferent connections re- mains to be explained, it is possible that the enhanced synaptic drive observed in the deprived M-cell may represent an increased sensitivity to synaptic drive resulting from an enhanced sensitivity to synaptic drive resulting from afferent inputs to the neuron. (Supported in part by NIH Grant No. NS-12132)
PSYCHO-PHARMACOLOGY
1541 EFFECTS OF INTRACRANIALY ADMINISTERED PENTYLENETETRAZOL ON ELECTROCOGGRAM AND CONCOMITANT MOTOR ACTIVITY IN THE FREELY MOVING RAT. Bruce J. Albala and Tibor Palffy. Skytop Labs. Division of Biopsychology, Syracuse Univ. Syracuse, New York 13210.

Pentyleneetrazol (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 characteristics 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 3H-PTZ in the brain following intracranial ( I.C. ) or intraperitoneal ( I.P. ) injections are comparable, the amount of 3H-PTZ is significantly lower following the I.P. administration. Electroencephalograms ( EEG ) recorded following I.P. or I.C. injection of PTZ indicate brain seizures that are similar qualitatively to those obtained following a subsequent dose of AM. These data suggest a dissociation between neural activity measured by EEG 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-helium at 1.0 to 1.5 atmospheres absolute (ATA) antagonizes ethanol depression without accelerating ethanol metabolism. The present experiments further investigated the utility of low level hyperbaric 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 1.0, 6.0 or 8.0 ATA with helium-oxygen mixtures adjusted to maintain equal partial pressure of oxygen-helium. Control animals were treated similarly and exposed to air (1.0 ATA) or 20% oxygen-helium (1.0 or 6.0 ATA). Immediately after return of their righting reflex, electromyograms were recorded in mg in deciliters are shown in parenthesis with the respective oxygen content of the mixture. Hyperbaric helium-oxygen significantly reduced sleep-time at 6 ATA (X(3.110) = 8.2 ± 0.8; X(0.20) = 10.3±2.6) and 8 ATA (X(2.602) = 8.8±2.3) when compared to 1ATA controls (X(0.20) = 18.0±3.0; X(3.110) = 17.7±2.9) (P<0.05). The 1ATA treatment did not significantly reduce sleep-time (X(0.20) = 17.2±3.6). Blood ethanol concentrations were significantly higher at 6 ATA (X(3.110) = 38.8±12) and 8 ATA (X(2.602) = 38.1±10) when compared to 1ATA controls (X(0.20) = 35.0±7; X(3.110) = 33.8±8). 2-tailed t-test. No significant difference was seen at 6 ATA (X(0.20) = 34.2±11) or 10 ATA (X(2.602) = 36.0±8). The present findings replicate the ethanol antagonism demonstrated in previous hyperbaric studies with pure oxygen and 20% oxygen-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. W. Andropoul* and L. S. Sklar*. (SPONSOR: 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 i-Dopa (400-600 mg/kg) administered prior to either inescapable shock or testing, eliminated the escape deficits otherwise observed. Moreover, pairing K-methyl-p-tyrosine (125 mg/kg) or bis (4-methyl-1-homopiperazinylthiocarbonyl) disulfide (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 thought 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.


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 prior to TP stressor. Previous results suggested that the augmentation of subsequent stress elicited amine changes. We now report that when TP sufficient to induce eating (approximately 80-100 psi) was applied daily for a total of 56 minutes 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.

The long-lasting influence of stress on the behavioral effects of amphetamine and haloperidol appears to be due to changes in endogenous norepinephrine and dopamine levels. The present findings particularly indicate that moderate stress can have a long-lasting, sensitizing influence on the organism.

Supported by USPHS grant MB 2414 to S.M.A.
LACK OF EFFECT OF SELECTIVE SEROTONIN DEPLETION ON BODY WEIGHT AND AMPHETAMINE ANOREXIA. L. A. Baez, R. A. Browning and M. Cuesta. Department of Psychology and School of Medicine, Southern Illinois University, Carbondale, IL 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) has been 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 investigated further by examining 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.) following injection. 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. The lack of a body weight effect in animals at a broad range of post-treatment intervals as well as the lack of a body weight effect in animals chronically depleted of brain 5-HT indicates the number of animals in each group.

Time after

Strain

Treatment

Weight ± S.E.M.

Vehicle

Long-Evans

24 days

37619 (6)

3636 (10)

Long-Evans

34 days

3595 (12)

3710 (10)

Sprague-Dawley

30 days

3268 (7)

3317 (7)

Long-Evans

1 year

5801 (6)

578 (17)

In addition, body weight data were obtained for eight weeks, at weekly intervals, in both male and female Long-Evans rats. As in previous experiments, there were no significant differences between the treated and untreated rats within each sex category. 5,7-DHT treatment also failed to alter the body weight. 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. 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 neurones exert a major inhibitory influence on ingestive behavior and body weight regulation.


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 of rats of amphetamine and phenylethylamine with other CNS stimulants. 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 5 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 pinazoline 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. Thoridazine, another antipsychotic agent with a low index of extrapyramidal side effects, selectively blocked phenylethylamine stereotypy. Two antidepressants, phenelzine and phenoxymethamphetamine, antagonize phenylethylamine stereotypy, whereas only phenelzine antagonizes cocaine and neither of these agents antagonize stereotypy induced by d-amphetamine. Methysergide, selectively blocks only phenylethylamine stereotypy. Although all three compounds, phenylethylamine, d-amphetamine and cocaine can produce extrapyramidal toxic paroxysmal movements in man, only phenylethylamine-induced behavior, as shown in our animal model, can be effectively blocked by antipyschotic agents.

EFFETS OF NALOXONE ON ACTIVITY AND REACTIVITY IN THE RAT. Gary G. Bertnson, 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, endorphins and enkephalins, can produce 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 preceded by 1) no treatment, 2) a 3 min. warm water (25°C) swim, and 3) a stressful 3 min. cold water (0°C) swim. There was no interaction between the stress condition and the time of measurement. The naloxone data provide further evidence that tonic activity exists in opioid peptide systems, and that these systems may participate in natural behavioral regulation.

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 vocalization. Cooperating rhesus monkeys (Macaca mulatta) in recent physiological 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 kg) on the tail of the subject in a manner which almost invariably induces behavior in awake animals, increased, and often doubled the firing rate of extra-cellularly 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 NSDA cells, our data also suggest that 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 NSDA neurons, 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 effects on NSDA activity. The stimulus used was vaginal-cervical pressure, since this stimulus inhibits active motor responses (Komiurak, 1974), and blocks tail pinch-induced vocalization (Bunney and Aghajanian, in preparation and our own unpublished observations).

A reciprocal relationship between tail pinch and cervical probing is further supported by the observation that while tail pinch is enhanced by drugs which block DA function, the same drugs potentiate some of the behavioral effects of cervical stimulation (Crowley et al., 1977). That is, there is a strong positive 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 over the onset of tail pinch. Continuation of the tail pinch after removal of the rod again activated the cell whereas cervical stimulation, by itself, produced little effect. This study appears to represent the first demonstration that stress can increase the firing of verified NSDA neurons.


Evidence from both the clinical and experimental animal literature suggests that sex and/or hormonal conditions 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 possibility 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 (Mislou and Fridhoff, 1973).

In the present study, the putative influence of estradiol 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 estradiol may exert a behaviorally relevant influence on DA function, its effects are likely to be highly dependent on the specific drug, dose, and conditions used. The finding that EB drastically altered the short-term (5-10 min.) development of catalepsy in response to the putatively administered spiroperi­ dol (20 ug/25 s.i.) suggests a central mechanism as well. The foregoing suggests that estradiol's effect on spiroperidol-induced catalepsy is probably dependent on the interaction of the drug with a neuroleptic-induced alteration in brain DA activity. In addition, it is possible that estradiol could contribute to some of the changes observed in experimental animals. The additional finding that EB increased the intensity of amphetamine (3 mg/kg)-induced stereotypy, when compared to OVX/oil treated controls, is consistent with this hypothesis.

These results suggest that estradiol's effects on neuroleptic-induced catalepsy may be due to a specific interaction of estradiol with the dopaminergic neurotransmitter system.

The relative potency, any unusual physiological or behavioral effects, and the percentage of relative safety were determined for a series of Δ⁹-THC analogues with side chain hydroxylation. The profile of activity was determined using 130 CF-1 male mice, 29.2 ± 1.2 gms. by a slight modification of Levine's procedure (J. Pharm. exp. Ther., 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 given as a normal injection. The relative potency was determined by the minimum dose that would cause a significant decrease in spontaneous activity with marked depression.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Onset (Minutes)</th>
<th>Peak (Minutes)</th>
<th>Duration (Minutes)</th>
<th>Relative Potency (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'-THC</td>
<td>4.0</td>
<td>1</td>
<td>15</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>1'-OH-Δ⁹-THC</td>
<td>2.0</td>
<td>2</td>
<td>10</td>
<td>60</td>
<td>2.0</td>
</tr>
<tr>
<td>1'a-OH-Δ⁹-THC</td>
<td>24.0</td>
<td>5</td>
<td>15</td>
<td>20</td>
<td>0.17</td>
</tr>
<tr>
<td>2'-OH-Δ⁹-THC</td>
<td>64.0</td>
<td>10</td>
<td>45</td>
<td>80</td>
<td>0.06</td>
</tr>
<tr>
<td>2'-OH-Δ⁹-THC</td>
<td>16.0</td>
<td>0.5</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>3'-OH-Δ⁹-THC</td>
<td>0.4</td>
<td>0.5</td>
<td>10</td>
<td>10</td>
<td>10.0</td>
</tr>
<tr>
<td>4'-OH-Δ⁹-THC</td>
<td>16.0</td>
<td>0.5</td>
<td>10</td>
<td>60</td>
<td>0.25</td>
</tr>
<tr>
<td>5'-OH-Δ⁹-THC</td>
<td>25.0</td>
<td>2</td>
<td>10</td>
<td>24</td>
<td>0.17</td>
</tr>
<tr>
<td>7'-OH-Δ⁹-THC</td>
<td>0.4</td>
<td>0.5</td>
<td>10</td>
<td>10</td>
<td>10.0</td>
</tr>
</tbody>
</table>

The relative potency varied slightly if hypersensitivity to tactile stimuli, crouched posture and gait, a somatosensor 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 40% in rectal temperature. This occurred at 25-30 minutes with essentially normal temperature for all except 1'-OH-Δ⁹-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 Δ⁹-THC analogues did not change significantly in their properties having an ataxic gait rather than the Δ⁹-THC narcotic type gait. 2'OH Δ⁹-THC and 5'OH analogues had a change in their properties having an acute increase in spontaneous activity with marked depression.

Reversal and Prevention of Acute Morphine Induced Catalepsy by Phenytoin in Female 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, and in other species, 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 involved in acute induced morphine catalepsy, phentoin, 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 (Kushinsky and Hornykiewicz, Eur. J. Pharmacol. 19: 119-122, 1972). A test was performed during 10 minutes of motor immobility. Experimental protocol consisted of the intravenous administration of: 1. morphine sulfate in saline, 2 mg/kg (a dose adequate to produce catalepsy for more than 2 hours); 2. phenytoin 35 mg/kg (all two animals were administered in a carrier of 40% propylene glycol and 10% ethanol); 3. morphine followed by phenytoin; 4. phenytoin followed fifteen minutes later by morphine; 5. saline alone; 6. phenytoin carrier alone; 7. phenytoin carrier followed by saline; and 8. saline 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 alone 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.


Female Sprague-Dawley rats, trained on the rotated (RR), wmn led floor grid chamber containing penobarbitonal (PB; 2.0 mg/g chow) and were given twice-daily i.p. injections (30 mg/g 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 immersion in 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/g 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/g PB, we found that chronic PB-treated rats had significantly higher levels of PB 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.)


Exploration away from the mother was studied in male offspring of female rats chronically exposed to lead (40 mg/g) via their drinking water from two weeks prior to breeding until their pups were weaned. The mean blood lead levels of the lead-treated pups at 21 days of age was 56±100 ml. + 3.4 E. U. Pup exploration away from the dam 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 (Macauley and Bull, Fed. Proc. 37:2764, 1978).
1559 CAUDATE LESIONS CHANGE THE BEHAVIORAL EFFECTS OF MORPHINE IN CATS.
Michele A. Canetta and Luis A. Baez. Dept. Psych., S. Illi. U., Carbondale, IL 62901
It has been reported that morphine-induced behavior is effectively antagonized with reserpine but not by AMPT pretreatment; the reverse is true of amphetamine behavior produced by amphetamine (Scheel-Kruger, Psychiat. Neurol. Neurosurg., 1972). The findings of this study are consistent with this interpretation. Rats were tested in a differential release of stored versus newly-synthesized catecholamines, respectively. This apparent difference in mechanism of action led us to conclude that dopamine exists in two different states in the neural system in addition to other differences in the neurochemical actions of these stimulants.

Previous research in our laboratory has demonstrated that a very low dose of the dopaminergic 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 morphine.

Adult male Long-Evans rats were placed in stabilimeters and allowed to habituate for 30 minutes. Following habituation, animals were divided according to the laterality of amphetamine-induced locomotion and stereotypy (Baez, Kerns & Smith, Eur. J. Pharm., 1977). Both the intermediate and high doses of morphine were effective in preventing the appearance of morphine-induced locomotion and stereotypy, even with the highest dose of morphine examined. These data suggest that morphine produces its behavioral stimulation via an enhancement of dopaminergic synaptic transmission. In this respect, morphine and amphetamine appear to be functionally similar.
The relationship between chronic marijuana use (≥ 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 and contraceptives, and were 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, androstenedione, 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.

CHANGES IN TELEENCEPHALIC SELF-STIMULATION RATE RESULTING FROM PRIOR SELF-STIMULATION EXPERIENCE AND FROM CHRONIC HALOPERIDOL. David C. Douglas 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 dependence that arise spontaneously or as a result of long term drug treatments. Nine rats were prepared with intracranial self-stimulation (ICSS) electrodes at telencephalic (9) or diencephalic (frontal cortex, MFC, or caudate nucleus, CN) and 7 of these animals also had successful placements in more caudal (CDL) points (lateral hypothalamic, LH, submaxillary nuc, and midbrain reticular formation, RF). RF 10-minute sessions were carried out with Cul ICSS either immediately before or after a 10-minute session of CDL ICSS. Parameters were: 0.1 nC negative pulses at monopolar electrode; 100 pulses/sec; 0.2 sec train; 30 sec inter-train; 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.

Although chronic administration of amphetamine in rats for a month (0.013 mg/ml) caused a apparent decrease in TEL ICSS; following withdrawal, ICSS recovered to predrug level in 3 cases, to 50-100% above baseline in one case, and to below (half) predrug rate in 5 cases (3MHC, 2CN). In four of these last cases, reduction of current intensity on 1-3 occasions led to a clear increase in ICSS rate. Since similar results were not found in drug it is hard to say whether this represents a drug-induced heightened sensitivity of the stimulated points. We have a small amount of data for male and female rats during and after drug treatment. 5 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 last rat showed a return of licking behavior to lower predrug levels after 31 injections of the same dose. It is hard to say whether this represents a drug-induced heightened sensitivity of the stimulated points. We have a small amount of data for male and female rats during and after drug treatment. 5 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. Supported by USPHS grant MH 24114 to S.M.A.

SOCIETY FOR NEUROSCIENCE

1562

CHANGES IN TELEENCEPHALIC SELF-STIMULATION RATE RESULTING FROM PRIOR SELF-STIMULATION EXPERIENCE AND FROM CHRONIC HALOPERIDOL. David C. Douglas 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 dependence that arise spontaneously or as a result of long term drug treatments. Nine rats were prepared with intracranial self-stimulation (ICSS) electrodes at telencephalic (9) or diencephalic (frontal cortex, MFC, or caudate nucleus, CN) and 7 of these animals also had successful placements in more caudal (CDL) points (lateral hypothalamic, LH, submaxillary nuc, and midbrain reticular formation, RF). RF 10-minute sessions were carried out with Cul ICSS either immediately before or after a 10-minute session of CDL ICSS. Parameters were: 0.1 nC negative pulses at monopolar electrode; 100 pulses/sec; 0.2 sec train; 30 sec inter-train; 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.

CHANGES IN TELEENCEPHALIC SELF-STIMULATION RATE RESULTING FROM PRIOR SELF-STIMULATION EXPERIENCE AND FROM CHRONIC HALOPERIDOL. David C. Douglas 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 dependence that arise spontaneously or as a result of long term drug treatments. Nine rats were prepared with intracranial self-stimulation (ICSS) electrodes at telencephalic (9) or diencephalic (frontal cortex, MFC, or caudate nucleus, CN) and 7 of these animals also had successful placements in more caudal (CDL) points (lateral hypothalamic, LH, submaxillary nuc, and midbrain reticular formation, RF). RF 10-minute sessions were carried out with Cul ICSS either immediately before or after a 10-minute session of CDL ICSS. Parameters were: 0.1 nC negative pulses at monopolar electrode; 100 pulses/sec; 0.2 sec train; 30 sec inter-train; 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.

CHANGES IN TELEENCEPHALIC SELF-STIMULATION RATE RESULTING FROM PRIOR SELF-STIMULATION EXPERIENCE AND FROM CHRONIC HALOPERIDOL. David C. Douglas 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 dependence that arise spontaneously or as a result of long term drug treatments. Nine rats were prepared with intracranial self-stimulation (ICSS) electrodes at telencephalic (9) or diencephalic (frontal cortex, MFC, or caudate nucleus, CN) and 7 of these animals also had successful placements in more caudal (CDL) points (lateral hypothalamic, LH, submaxillary nuc, and midbrain reticular formation, RF). RF 10-minute sessions were carried out with Cul ICSS either immediately before or after a 10-minute session of CDL ICSS. Parameters were: 0.1 nC negative pulses at monopolar electrode; 100 pulses/sec; 0.2 sec train; 30 sec inter-train; 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.

CHANGES IN TELEENCEPHALIC SELF-STIMULATION RATE RESULTING FROM PRIOR SELF-STIMULATION EXPERIENCE AND FROM CHRONIC HALOPERIDOL. David C. Douglas 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 dependence that arise spontaneously or as a result of long term drug treatments. Nine rats were prepared with intracranial self-stimulation (ICSS) electrodes at telencephalic (9) or diencephalic (frontal cortex, MFC, or caudate nucleus, CN) and 7 of these animals also had successful placements in more caudal (CDL) points (lateral hypothalamic, LH, submaxillary nuc, and midbrain reticular formation, RF). RF 10-minute sessions were carried out with Cul ICSS either immediately before or after a 10-minute session of CDL ICSS. Parameters were: 0.1 nC negative pulses at monopolar electrode; 100 pulses/sec; 0.2 sec train; 30 sec inter-train; 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.
1565 SILASTIC TUBING SYSTEMS FOR CONTINUOUS DRUG ADMINISTRATION: CONSTRUCTION AND APPLICATIONS. Michael S. Eisen, 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 stereotypes 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 remarkably 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.


Use of a new sensitive motility transducer, which allows reduction of movement to power spectra, has demonstrated a number of drug-induced changes which cannot be quantified with 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 Eisen, 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 aberrant 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 stereotypes evoked by amphetamine or apomorphine injections just after amphetamine pellet removal, whereas daily injections of the same amount of amphetamine produce heightened stereotypes. 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. Kometsky, 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 & Kometsky, Science, 195:189, 1977; Esposito, Motola & Kometsky, 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 high doses. The behavior of each subject was analyzed to determine the effects of these agents.
GENETIC DIFFERENCES IN DOPAMINE-MEDIATED SPONTANEOUS AND DRUG-ELICITED BEHAVIORS IN INBRED MOUSE STRAINS WITH DIFFERENT NUMBERS OF MIDBRAIN DOPAMINE NEURONES. E. S. Fish, T. H. Joh and D. L. Reis, Laboratory of Neurobiology, Dept. Neurology, Cornell Univ. Medical College, New York, N.Y. 10021

Pole of catecholaminergic receptors in the hypothalamic control of lordotic behavior in the ovariectomized estrogen primed rat. Mark H. Foreman* and Robert L. Moss, Dept. of Physiol. and Obstet. of Anesthesia, Peter Bent Brigham Hosp., and Dept. of Psychiatry Harvard Medical School, Boston, Mass. 02115

Comparison of dopaminergic, α- and β-adrenergic receptor stimulants and blockers with unilateral, 23 gauge stainless steel cannulas implanted into either the medial preoptic area (MPA), arcuate nucleus (ARC), or lateral hypothalamic area (LHA). In the first experiment, OX rats were primed with 100-250 µg of estrone 48 hrs prior to testing to maintain low proinflammatory reactivity (mean lordosis to mean ratio, L/H = 1.64). The infusion of 200 or 8000 µg of dopamine, norepinephrine, epinephrine, dopaminergic receptor stimulant, amphetamine, α-adrenergic receptor blockers, phentolamine or phenoxybenzamine, produced a similar, isoxysotrenol 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 500µg of estrone to maintain low proinflammatory reactivity (mean L/H = 0.88). MPOA or ARC-M infusions of dopaminergic receptor blocker, haloperidol, and α-flupenthixol; adrenergic receptor blocker, propranolol; or α-adrenergic receptor stimulant, methoxamine significantly decreased mating behavior compared to vehicle (p < .001). However, MPOA or ARC-M infusions of amphetamine, isoxysotrenol, phentolamine, or phenoxbenzamine had no significant effect.

A third experiment evaluated the hypothalamic interactions between catecholaminergic and dopaminergic mechanisms. Responses were made among both dopaminergic responses to 0.5 µl MPOA and ARC-M infusions of LHRH (500 µg); LHRH (500 µg) + haloperidol (1.0 µg); LHRH (500 µg) + methoxamine (1.0 µg) and vehicle in OX rats primed with 100-250 µg estrone. Infusions of LHRH into either area significantly increased lordotic behavior and increased lordosis in the presence of haloperidol, propranolol or methoxamine abolished this response. These results suggest that MPOA and ARC-M areas contain neurotransmitters which contribute to the mediation of lordotic responses and can amplify sexual behavior in response to dopaminergic, α- and LHRH receptor stimulation and α-receptor blockade. The dopaminergic and β-adrenergic blockade or α-adrenergic stimulation can depress either estrogen- or LHRH-facilitated mating behavior.

Supported by NSF Grant POM76-10015.


Potential of inhalational anesthetic agents is generally expressed as an ED50 or ED100, or rate of withdrawal 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 of analgesic and anesthetic properties are not possible. We sought a measure of graded behavioral effect that would obviate this problem by quantitating the degree of narcosis. The results of the Long-Evans strain of rats were examined. Rats were primed with 1.0 µl doses of dopamine, norepinephrine, epinephrine, dopaminergic receptor stimulant, amphetamine, as well as α-adrenergic receptor blockers, haloperidol and propranolol, or α-adrenergic receptor stimulant, isoxysotrenol, 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 500µg of estrone to maintain low proinflammatory reactivity (mean L/H = 0.88). MPOA or ARC-M infusions of dopaminergic receptor blocker, haloperidol, and α-flupenthixol; adrenergic receptor blocker, propranolol; or α-adrenergic receptor stimulant, methoxamine significantly decreased mating behavior compared to vehicle (p < .001). However, MPOA or ARC-M infusions of amphetamine, isoxysotrenol, phentolamine, or phenoxbenzamine had no significant effect.

A third experiment evaluated the hypothalamic interactions between catecholaminergic and dopaminergic mechanisms. Responses were made among both dopaminergic responses to 0.5 µl MPOA and ARC-M infusions of LHRH (500 µg); LHRH (500 µg) + haloperidol (1.0 µg); LHRH (500 µg) + methoxamine (1.0 µg) and vehicle in OX rats primed with 100-250 µg estrone. Infusions of LHRH into either area significantly increased lordotic behavior and increased lordosis in the presence of haloperidol, propranolol or methoxamine abolished this response. These results suggest that MPOA and ARC-M areas contain neurotransmitters which contribute to the mediation of lordotic responses and can amplify sexual behavior in response to dopaminergic, α- and LHRH receptor stimulation and α-receptor blockade. The dopaminergic and β-adrenergic blockade or α-adrenergic stimulation can depress either estrogen- or LHRH-facilitated mating behavior.

Supported by NSF Grant POM76-10015.


Potential of inhalational anesthetic agents is generally expressed as an ED50 or ED100, or rate of withdrawal 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 of analgesic and anesthetic properties are not possible. We sought a measure of graded behavioral effect that would obviate this problem by quantitating the degree of narcosis. The results of the Long-Evans strain of rats were examined. Rats were primed with 1.0 µl doses of dopamine, norepinephrine, epinephrine, dopaminergic receptor stimulant, amphetamine, as well as α-adrenergic receptor blockers, haloperidol and propranolol, or α-adrenergic receptor stimulant, isoxysotrenol, 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 500µg of estrone to maintain low proinflammatory reactivity (mean L/H = 0.88). MPOA or ARC-M infusions of dopaminergic receptor blocker, haloperidol, and α-flupenthixol; adrenergic receptor blocker, propranolol; or α-adrenergic receptor stimulant, methoxamine significantly decreased mating behavior compared to vehicle (p < .001). However, MPOA or ARC-M infusions of amphetamine, isoxysotrenol, phentolamine, or phenoxbenzamine had no significant effect.

A third experiment evaluated the hypothalamic interactions between catecholaminergic and dopaminergic mechanisms. Responses were made among both dopaminergic responses to 0.5 µl MPOA and ARC-M infusions of LHRH (500 µg); LHRH (500 µg) + haloperidol (1.0 µg); LHRH (500 µg) + methoxamine (1.0 µg) and vehicle in OX rats primed with 100-250 µg estrone. Infusions of LHRH into either area significantly increased lordotic behavior and increased lordosis in the presence of haloperidol, propranolol or methoxamine abolished this response. These results suggest that MPOA and ARC-M areas contain neurotransmitters which contribute to the mediation of lordotic responses and can amplify sexual behavior in response to dopaminergic, α- and LHRH receptor stimulation and α-receptor blockade. The dopaminergic and β-adrenergic blockade or α-adrenergic stimulation can depress either estrogen- or LHRH-facilitated mating behavior.

Supported by NSF Grant POM76-10015.
ASSOCIATIVE AND NONASSOCIATIVE EFFECTS OF D-LYSYRIC ACID DIETHYLWANIDE (LSO) ON PAVLOVIAN AVERSIVE AND APPETITIVE CONDITIONING IN THE RABBIT. A. Gormezano & 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 μmol/kg LSO. In the present study examined whether LSD would produce enhancement in Pavlovian appetitive conditioning of the rabbit jaw movement response. For purposes of contrast two experimental groups were compared each involving the rabbits' training to tone and light and conditioned stimulus (CSs) at a CS-UCS interval of 800 msec. For one experiment, involving conditioning of the jaw movement response, the second experiment involved conditioning of the rabbit jaw movement response, utilized a 3a, 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-US interval. Animals were intravenously administered with equal volumes (0.6 ml/kg) of vehicle or LSD (30 mmol/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 responses relative to vehicle controls. Enhanced acquisition occurred to both the auditory and visual CSs. Moreover, the enhancement in the rate of acquisition produced by LSD was reflected not only in the greater overall level of conditioned responding to the CS 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 conditions. These procedures indicated 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 appetitive 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.


Female Long-Evans rats were given solutions of barbitail (1 mg/ml) or chlordiazepoxide HCI (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 (H2O group). Another group was given a liquid diet; the control group was pair-fed a diet in which sucrose was substituted isocalorically for ethanol (sucrose group). All treatments were begun 1 week before the pregnant female rats were mated with white male rats. The 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:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Litter Size</th>
<th>Body Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbitail (vs. H2O)</td>
<td>NC NC</td>
<td>NC NC NC NC</td>
</tr>
<tr>
<td>CDP (vs. H2O)</td>
<td>NC NC</td>
<td>NC NC NC NC</td>
</tr>
<tr>
<td>Ethanol (vs. Sucrose)</td>
<td>NC NC</td>
<td>NC NC NC NC</td>
</tr>
<tr>
<td>Sucrose (vs. H2O)</td>
<td>NC NC</td>
<td>NC NC NC NC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquisition Latencies</th>
<th>FR20 Rates</th>
<th>p&lt;0.05</th>
<th>p&lt;0.01</th>
<th>NC, no significant change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbitail (vs. H2O)</td>
<td>*** ***</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>CDP (vs. H2O)</td>
<td>** **</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Ethanol (vs. Sucrose)</td>
<td>** **</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

The effects of ethanol avoidance behavior resembled those of CDP but not those produced by barbitail. These alterations in avoidance responding may be due to the 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 barbitail 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 ethanol exposure, barbitail or both (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.
Iprindole is a potent enhancer of spontaneous and KCl-induced efflux of norepinephrine from rat brain slices.

Iprindole is an atypical tricyclic antidepressant whose clinical efficacy 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 received psychoactive medication for years were interviewed. 21 of them showed evidence of Tardive Dyskinesia symptoms. (25%) Iprindole is a potent enhancer of spontaneous and KCl-induced efflux of norepinephrine from rat brain slices, at doses of iprindole as low as 10 picomolar. Maximal enhancement was seen at 37°C when the slices were incubated with 10 μM iprindole and 10 μM tropolone, 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 μM of washed slices in 10 ml of Krebs HCO3, Ringer containing nialamide and tromolone, 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 dependent on temperature. As high as 50 mM KC1 caused a loss of only 4% of the radioactivity in the slices at 0°C as compared with 70% loss at 37°C in this system. Iprindole increased spontaneous efflux as well as efflux induced by the addition of KCl (20 to 75 mM) as long as control efflux (no iprindole present) was below 50% of the radioactivity curve. Above the 50% range, the efflux was not increased. Significant increases in both types of efflux (50% above control and maximal enhancement) were observed at 10 μM iprindole. These findings emphasize that although iprindole is not as potent as other tricyclic antidepressants in inhibiting NE uptake, its marked potency in enhancing NE efflux would increase the availability of NE during nerve transmission, in agreement with the theory of the role of norepinephrine as a modulator of neurotransmitter functions.


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 intraperitoneal administration of saline or of 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 effect; an initial phase of behavioral depression followed by a sustained behavioral stupor associated with high-voltage EEG slow-wave activity (slow bursts) followed by a secondary phase of behavioral arousal and locomotion. EEG activity and spontaneous movement were observed during the acute phase of behavioral depression. The authors would like to express their gratitude to Dr. Leitschung, his colleague and many others who made this study possible.
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 V980, Chicago, Ill. 60680.

Rats working for electrical stimulation of the lateral hypothalamic 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 independent 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 physically 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 'B' at one end having a response profile very similar to the hypothalamic animals, and type 'A' 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) stimulus at the hypothalamic level. In any case, a depression in rate 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.

ROTATIONAL BEHAVIOR AFTER ACUTE AND CHRONIC SYSTEMIC AMPHETAMINE TREATMENT. L. Kook (10 min. time-locked to SRON: M.G. Webster). Department of Psychology, University of Saskatchewan, Saskatoon, Sask., Canada. Rotating behavior 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 6-methyl-2-pyridone-5-carboxylic acid (MPD) 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 the 4-methyl-1-homopiperazinylthiocarbonyl disulphide (FLA-63) successfully antagonized the drug-induced circling, without altering the locomotor excitation. Pretreatment with pcprominal, a B-adrenergic receptor blocker, decreased the circling behaviour induced by amphetamine, whereas phenoxybenzamine, an a-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 pcprominal 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 in selective attention elicited by acute and chronic amphetamine treatment.

HALOPERIDOL CLASSICAL CONDITIONING - PARADOXICAL RESULTS. James J. King*, Stanley R. Schiff* and Wagner H. Bridge, 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 sessions) and pseudo-conditioning control groups (saline as treatment for experimental session, followed by drug injection 45 min to 1 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 quadrants 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.

EFFECTS OF ETHANOL ON PUNISHED RESPONDING. C. F. Koob, S.L. Foote, and F. F. 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 chlorodiazepoxide (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, chlorodiazepoxide 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 chlorodiazepoxide 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 produces effects on cognitive properties similar to the benzodiazepines and that both drugs interact with a naloxone sensitive substrate.

Supported by grant AA 03504.
SOCIETY FOR NEUROSCIENCE

1585


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 2 male outpatients taking thiothixene (mean dose:208mg/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), then matched placebo for 4 weeks (acute) and returned to baseline dose for 4 weeks (acute). An AN fasting blood sample was obtained at each weekly visit along with a Brief Psychiatric Rating Scale (BPRS) and level of Stock of Mood States (POMS). Samples from the same subject were measured in the same radioimmunoassay.

The mean prolactin during placebo was 5.8 ± 0.5 ng/ml and differed from the chronic (12.2 ± 2.2 ng/ml) and acute (14.6 ± 2.1 ng/ml) prolactins (F = 6.8, p < 0.01). Nine patients had higher mean prolactines during the acute than chronic period (six week test, p < 0.05). The percent increases in prolactin from chronic to acute period ranged from 4 to 120 percent (mean = 62%).

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 (< 4 ng/ml) did show the greatest change in POMS scores ( > 50%) after drug withdrawal (Fisher Exact Test, p = 0.006). There was a trend for lower prolactins during placebo to be associated with clinical worsening during placebo (BPRS: p = 0.067; POMS: p = 0.067). 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 = 0.006). 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 = 0.067; POMS: p = 0.013) and greater improvement after drug resumption (BPRS: p = 0.013; POMS: p = 0.006). Prolactin levels, tolerance and clinical state were not related to dose or duration of drug treatment.

Either dopaminergic receptor sensitisation 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 3H-NEUROLEPTIC BINDING IN POST-MORTEM SCHIZOPHRENIC BRAINS. Tyrone Lee and Philip Seemman. Pharmacology Department, University of Toronto, Toronto, Canada.

It has been reported in a previous communication (Lee and Seemman, Proc. Neurosci. Soc. 3[44], 1977) that 3H-haloperidol receptors in the caudate nucleus, putamen and nucleus accumbens were 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 brains from schizophrenic 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.K. Tourelottie (NINCDS/NIH Human Neurospecimen Bank, Los Angeles, California), Dr. K.D. Bird (MRC Human Brain Bank, Cambridge, England) and Dr. O. Hornykiewicz (Vienna, Austria). Tissues were homogenized in Tris-buffer, incubated with 3H-apomorphine, 3H-haloperidol and 3H-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 3H-haloperidol, 3H-spiroperidol and 3H-apomorphine (fmol/mg protein) to normal and schizophrenic brain tissues were as follows:


1587


The selective depletion of brain dopamine (DA) by intracisternal administration of 6-OHDA in the 3 day old rat pup results in a spectrum of behaviors including hyperreactive 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 behavior and can reverse the learned helplessness in developing rat pups treated with 6-OHDA. 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 15% 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 rats pups APO at both dosages reduced escape latency by 16% (p < 0.001).

In both normal and 6-OHDA treated rats, APO induced a dose dependent fashion and these effects were more pronounced as maturation progressed. Thus APO increased activity by only 40% at 10 days but by 200% at 19 and 26 days. APO increased activity by 6 fold in both normal and 6-OHDA treated rats at 19 and 26 days. During chronic treatment with 6-OHDA, APO decreased activity by 50% at 19 and 26 days, and returned to baseline activity in 6-OHDA treated rats (p < 0.001).

The effects of APO on schedule induced and schedule dependent behavior were dose and schedule dependent. APO increased activity by 4 fold in both normal and 6-OHDA treated rats during the acute phase revealed a significant decrease in lever presses and water consumption for at least ten days. During the acute phase, animals were injected with 0.5% saline, 2, 4, 8 and 16 mg/kg of imipramine in a counterbalanced design. Following return of all the measures to pre-injection baseline, the same animals were injected with 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. 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.

1588

ENHANCED 3H-NEUROLEPTIC BINDING IN POST-MORTEM SCHIZOPHRENIC BRAINS. Tyrone Lee and Philip Seemman. Pharmacology Department, University of Toronto, Toronto, Canada.

It has been reported in a previous communication (Lee and Seemman, Proc. Neurosci. Soc. 3[44], 1977) that 3H-haloperidol receptors in the caudate nucleus, putamen and nucleus accumbens were 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 brains from schizophrenic 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.K. Tourelottie (NINCDS/NIH Human Neurospecimen Bank, Los Angeles, California), Dr. K.D. Bird (MRC Human Brain Bank, Cambridge, England) and Dr. O. Hornykiewicz (Vienna, Austria). Tissues were homogenized in Tris-buffer, incubated with 3H-apomorphine, 3H-haloperidol and 3H-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 3H-haloperidol, 3H-spiroperidol and 3H-apomorphine (fmol/mg protein) to normal and schizophrenic brain tissues were as follows:

| SCHIZOPHRENIC | CAUDATE | 46.4±1.9 (21) | 47.7±1.7 (16) | 28.7±2.8 (13) |
| | PUTAMEN | 45.9±3.3 (21) | 87.6±1.1 (12) | 36.3±2.7 (13) |
| | N. ACCUM. | 51.7±3.9 (21) | 30.5±3.1 (4) | 30.5±3.1 (4) |

* P < 0.001; ΨΨ non-significant.

(Number in parentheses denotes number of human brains assayed, each with 5-20 replicate determinations).
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, 3H-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 3H-muscimol were utilized, ethylene side chain 3H (12 Ci/mole, mus-A) and muscimol having a 3H on the 4 position of the isoxazole ring (10.35 Ci/mole, mus-B). Administration (5 μCi i.v.) of either mus-A or mus-B into mice resulted 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 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 3H-GABA, 3H-mus-A or 3H-phenobarbital indicated that, in terms of the percent of dose administered, muscimol accumulates in the brain to a greater extent than 3H-GABA, which is no more than an order of magnitude less than 3H-phenobarbital. Determination of chloroform/water partition coefficients revealed that muscimol and GABA have similar coefficients and that they are 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, USPSN RDA NS-00335 (S.E.E.) and (A.M.) a Salk Institute-Texas Research Foundation Fellowship.)

In vitro, ouabain is 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 by barium interdigitations. 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 0.1 mM 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 (rₛ = 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), and non-depressed control subjects (F = 19, p < 0.01). Moreover, although correlation substantially reduced the transmembrane Li⁺ distribution values in both control subjects (rₛ = 0.80, p < 0.05) and in patients with unipolar and secondary depression (rₛ = 0.79, p < 0.001), it did not add to the distribution parameter of the bipolar patient group (rₛ = 0.24, p > 0.1). These findings provide evidence that, with respect to Li⁺ transport, the membrane characteristics of cells may differ among those who are 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.

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 animals received chloridiazepoxide (CDP; 10 mg/kg) as base so that when CDP was administered during extinction it would not produce dis-salivation. Following acquisition, eleven of the lesion 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 running velocity (in v) of each animal was measured at the same 30 min interval prior to each session. The following results were obtained: 1. During acquisition of running, 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 perseverance of responding during extinction previously reported following selective re-duction of forebrain NE (e.g., Tremmel et al., Brain Res. 126: 159, 1977). 3. The two groups which received CDP were indistinguishable on measures of positive reinforcement. This result is contrary to the increased suppression of ongoing food reward observed by others. 4. Depletion of forebrain NE attenuated the anti-extinction effect of CDP. These 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 NE (i.e., 35%) in the DTB 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.


Phencyclidine (PCD) is a psychotomimetic drug of abuse which has recently been implicated in the production of a schizophrenia-like syndrome. The effects of PCD on behavior in rats are dose-dependent in a manner similar to that of other psychotomimetics. PCD is a reversible antagonist at the serotonin (5-HT) receptor complex and produces a dose-dependent increase in serotonin levels in the frontal cortex. The present study investigated the dose-response effects of PCD on stereotyped behavior in the rat. 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/hr) and 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-hour period (infusion rates ranged from 0.5-2 ml/hr). This infusion phase ethanol infusions were discontinued and rats were observed hourly for 20-hr and thereafter at 4-hr intervals for signs of physical dependence. All ethanol treated rats showed withdrawal symptoms (moderate to severe, n=9; mild, n=2). Saline treated rats showed no symptoms. The most reliable symptoms were listless (n=3), anoletic (n=2), and self-mutilation (n=1). None of the rats showed any signs of alcohol withdrawal. These data suggest that 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.


We have carried out in vivo studies of dopamine (DA) receptors in the brain of the rat using the 125I-labeled antagonist (+)butacloam (125I-SP) as a ligand (Life Sciences 22: 203, 1978). There was a regional distribution of stereospecifically bound 125I-SP consistent with in vitro studies and with known DA innervation of basal ganglia. The binding was saturable and was blocked by DA agonists and antagonists, such as (+)butacloam. Compounds inactive at DA receptors, such as (-)butacloam were ineffective in blocking 125I-SP binding. These data suggested that autoradiographic studies of the DA recep­tor be feasible. In the present work male rats were adminis­tered 100 mCi (45 pCi/mg) of 125I-SP (autoradiographic studies) of 3H-SP via tail vein, were decapitated two hours later and various brain regions were either analyzed for radioactivity or were prepared for autoradiography. Autoradiographic studies were found to be highly localized in specific brain regions. The olfactory tubercles and the nucleus accumbens were heavily labeled, consistent with 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 strainum 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 125I-SP as re-log (a) do not, such as the ventral pallidum, the nucleus accumbens or the substantia nigra. The nigrostriatal dopaminergic system has recently been implicated in the production of a schizo­phrenia-like syndrome. The effects of a-synuclein receptor antagonists on the DA nigrostriatal system will be presented. Supported by NIAAA Grants DA03562 and BS03562.

1596 INDUCTION OF PHYSICAL DEPENDENCE UPON ETHANOL IN RATS USING INTRAVENOUS INFUSION. Robert Hume and Anna M. Charles of 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. 20 min. after 0.9 ml/kg of saline (30% v/v) was infused over a 7-day period (inter-animal range 5-10 days) the rats were observed hourly for 20-hr and thereafter at 4-hr intervals for signs of physical dependence. All ethanol treated rats showed withdrawal symptoms (moderate to severe, n=9; mild, n=2). Saline treated rats showed no symptoms. The most reliable symptoms were listless (n=3), anoletic (n=2), and self-mutilation (n=1). None of the rats showed any signs of alcohol withdrawal. These data suggest that 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.

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 stereotypes illustrate that many elements of an animal's repertoire are interdependent, and amphetamine-induced stereotypes represent further stimulation of locomotor activity. We investigated the possibility that tolerance or sensitization may develop differentially to the locomotor and stereotypic 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 amogenital 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 l.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 reetermined and also, the effects of cocaine were assessed. Our results indicate that (1) tolerance does not develop to the anti-aggressive effects of d-amphetamine, (2) all subjects maintained mice show tolerance to the locomotion stimulating effect of the drug, (3) amphetamine induces stereotypes at a lower dose and to a greater degree in mice maintained in a chronic shape 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 determination 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.)


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 discriminative performance is not 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 90 mg/kg 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-FS 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 lever that was to be classically conditioned with 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 FR schedule 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.

EFFECT OF IPRINDOLE ON MOUSE BRAIN SEROTONIN METABOLISM. Bertha G. Ortega-Corona, José Carranza-Acevedo, Patricia Guzmán-Amaya*, Nora Esparza-Avalos*, Guadalupe Castro-Osuna*. Subjefatura de Investigación Básica, Instituto Mexicano del Seguro Social, P.O. Box 73-032, Mexico City, 73, D.F., MEXICO.

Tricyclic antidepressant drugs (TAD) were found to block tricyclic antidepressant 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 hypotheses. 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 determined by Welch and Welch's method and MAO activity was measured by Warman'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 decrease at all 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 serotonine metabolism.


Albinos 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, a genetic line comparison between albinos 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 c57bl/6J-c2J males 137-150 days of age (mean = 146) at the start of the experiment. Each pair was from a different litter and consisted of an albinos, c2J/c2J, and a pigmented, +/c2J, matched for weight. In some litters additional male mice were available and used as controls (+ albinos, + 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 l.p. with sodium pentobarbital (Nembutal); dosage increased 10 mg/kg/week (to 150 mg/kg as of 5-7-76). Controls were injected l.p. with saline 2 ml/kg. After injection littermates were placed together in observation cages. Controls were removed upon experimental animals' loss of righting reflexes (LRR). Time differences were found for the return of the righting reflexes (RRT). Experimental animals were returned to their home cages after RRT. Between experimental sessions littermates were housed together in the mouse colony. Median sleep-time (interval from LRR to RRT) was consistently greater for albinos at dosages greater than 50 mg/kg. Sleep-time differences were significant (P > .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 albinos and pigmented mice. No significant differences were found for the interval of RRR. Sleep-time results are consistent with the findings of Shearer et al. (1973), but LD50 data are not. However, their study involved comparisons between several strains of rats or mice whereas ours was a within strain comparison of mice.

Supported by NIH Grant No. 1 RO1 BY 01885-01 and Fight For Sight, Inc., (1971, Grant No. D-599.)
1601 AN OPIATE EXCESS MODEL OF CHILDMOOD 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 modeled in animals—a low incidence of crying during infancy, a failure to cling to parents, a lack of 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 animals 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 piglets, guinea pigs, and chickens in dose-dependent fashion. For instance, in 8-16 day old guinea pigs, 0.25 and 1.0 mg/kg morphine significantly reduced crying to 60% 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 press the lever and spend 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 social 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 nalbuphine.

Supported by Research Scientist Development Award MH-0086 to JP.


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 adenylyl cyclase activity in the ipsilateral caudate nucleus 24 hours after the injection. (Quenzer et al., Proc. Natl. Acad. Sci. USA 75: 78, 1978). We now find that adenylyl 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 weight/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 adenylyl cyclase activity in substantia nigra (23.4 pmol cAMP/mg tissue/3 min) is much lower than that in caudate but it too increased (46.9 pmol/mg tissue/3 min) 24 hrs after intranigral cholera toxin. Although the basal adenylyl 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 3 H and 100 µM substrate concentrations. The prolonged increase in adenylyl cyclase activity in combination with unchanged phosphodiesterase metabolism of cAMP suggests that endogenous levels of cAMP are elevated on the caudate 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 rats 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 later but is not 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.

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, Hoornstra & Campbell, JCPP, 1971, 77, 358-393). 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. Consistent 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 have 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.


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% 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 more 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.

We conclude that the receptor mediating the discriminative cueing property of nicotine possesses a high degree of geometrical specificity.

(Submitted by grants from the National Institute on Drug Abuse.)

The modulation by morphine of the aversive properties of electrical brain stimulation has been studied less extensively than the drug's direct effects on the parameters of stimulation. The present study was undertaken in order to extend previous findings in several ways. First, we used a frequency-threshold method to avoid using an amount of drug that would be too large to determine the contribution of performance effects that often bias response rate measures. Second, we attempted to establish task- and dose-dependent relations 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, 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 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 ephrine HCl injections was begun. An initial dose of 20 mg/kg (0.4 mg/kg base) was incremented in 0.1 log 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 have been related to the rate of threshold response to stimulation and escape at each placement.

Experiments are now in progress to determine concurrent changes in self-stimulation thresholds for withdrawal 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 drug-rewarding properties. This work was supported by Canadian MRN Grant A0308.


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 in infancy and then housed without a mother-absent treatment show no such diminished response in adulthood, indicating a crucial maternal component. The present study was undertaken to show that when normal-style maternal interactions are included with or without the mother-absent treatment, stressors (eliciting stress in the offspring) 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 effect of the donor pups by increasing their care-giving behavior. MULTIP mothers responded by decreasing their care-giving behavior. Both PRIMIP and MULTIP donor pups in a fashion intermediate to the STRZ and BSKT conditions, except that mothers exposed to ANES donor pups constructed better reared nests. Following weaning, these mothers with female offspring were subjected to six consecutive daily injections of saline, 2.5 mg/kg or 5.0 mg/kg of d-amphetamine 30-55 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 0.0 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.


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. We began this in mind, the following study was conducted. Following a baseline observation period, d-amphetamine (d-Amph), 1.6 mg/kg (0.4 mg/kg base) was administered in an i.p release form, every 12 hours for 12 consecutive days to 13 female stump-tailed macaques selected from 6 stable, adult, social colonies of 4-5 monkeys each. No more than 2 monkeys/group were present in the colony (Ta) during one i.p 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 Ta monkeys developed stereotypy and hypervigilance as abnormal behaviors. The form of stereotype varied between animals, but higher ranking (HR) monkeys displayed more social stereotype 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 by means of distinguishing social and non-social distance. LR monkeys became isolated earlier in Tx than HR monkeys. HR monkeys had increases in submissive gesture scores during Tx. Several HR female monkeys showed an increase in threat-grouping responses that were rarely followed with attacks. d-Amph-induced excessive scratching in 8 monkeys. Several of the behavioral changes induced in monkeys by d-Amph correlated with changes in monosodium glutamate (MAG) locomotion. We also determined 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.

BIPARSI 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 hydroxins 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 glycocytosis—derided and of G-6-P in NADH, of glycoglycys-derived AcCoA. Brain glucose levels would be expected to (and do) transiently rise after acute ethanol, followed by a decay in glucose levels in later, as ethanol is cleared from the body (Arch Int Pharmacol., 154, 108, 1965). Glycogen levels in brain transiently fall, indicating a greater rate of glycoxyn to glucose-6-phosphate (G-6-P) than of high-NADP-suppressed glucose to G-6-P.

It has been proposed that decreased total immediately mobilizable energy reserves in brain may underly susceptibility to amphetamine-induced seizures (Masions) (Res. Comm. Psychol., Psychiat., Behav., in press; Pharm. Bioch. Behav., 5 (5) in press, 1978). Should this model hold, then one would predict an early protection from SAGS by ethanol, followed by a transiently 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 audio-on priming at 16 days of age (127 ± 20 kg) 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 acemases, wild run) were discernible by 5 min post-infusion.

Mice were tested for SAGS at 30-min or 60-min intervals up to 7 hr. post-infusion. Data are shown below: (mice completely wild run=1, clonic- tonic=2, clonic-stereotypy=3, tonic-clonic=4, death=5).
BEHAVIORAL EVIDENCE FOR ANATOMICAL SPECIFICITY OF ACTION BY CLOZAPINE. Thomas F. Seeger* and Eliot L. Gardner, Depts. of Pharmacology, Psychiatry, and Neurosciences, Albert Einstein College of Medicine, Bronx, NY 10461

Biochemical evidence suggests that clozapine has a dopamine (DA) blocking activity specific to mesolimbic DA system, while "classical" neuroleptics such as haloperidol have equal potency in both the nigro-striatal and mesolimbic DA systems. However, our present evidence for this specificity is lacking, perhaps due to the need for a good behavioral assay for mesolimbic DA function.

We have studied the effect of chronic clozapine or 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 drug control (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 a significant increase in all behaviors in comparison to the clozapine 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-06949 and GM-07260)

LONG-LASTING BEHAVIORAL SEIZURES IN THE YOUNG CHICKEN IN-duced by phencyclidine. Clifford J. Sherry and F. Scott Hunter*. Biology Dept. Texas A & N 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, K. Anesthesia 1959, 31. 503) 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. Pharmacol. Exp. Ther. 1959. 127. 4). The ED50 for seizure development in the 2 day old chick is 43.5 mg/kg. When compared to the 2 day old chick, older chicks tended, on the average, to have more clonic seizures and an average of 4 minutes of tonic seizures. The overall age length of tonic seizures decreased. In older chicks, the average length of tonic seizures tended to increase, while the average frequency of clonic seizures decreased. When the dose level of PCP was increased to 10 or 15 mg/kg (the ICSS rats were exposed to 1, 5, 10, 20, 40, 60, or 30 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 on the back). The LD50 for PCP is the 2 day old chick is 43.5 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 an average of 4 minutes of tonic seizures. The overall age length of tonic seizures decreased. In older chicks, the average length of tonic seizures tended to increase, while the average frequency of clonic seizures decreased. When the dose level of PCP was increased to 15 mg/kg, the chicks showed an attenuated 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 (P47-54) testing intervals. Drug treated offspring did not differ from controls on any of these measures at the intermediate (P35-62) testing interval. Thus behavioral and pharmacological effects of chronic haloperidol treatment during development and adulthood may produce behavioral heterogeneity, the later behavioral responsiveness of some animals may vary: adult treated animals show an accentuated response to amphetamine and attenuated response to haloperidol, while the opposite is found in developmentally treated animals (Chen, G. et al., J. Pharmacol. Exp. Ther. 1959, 127. 479). 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.


Phencyclidine (PCP) is an anesthetic drug with psychotomimetic properties, which has been shown to effect brain dopaminergic, noradrenergic, cholinergic, and serotontic systems. Recent exeriments 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 experiment 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, stereotyped sniffing, and rearing, but not on circling behavior (for 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. Kontak, C. C. Loukis, M. J. Wayner, B. A. 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 a fundamentally different response from that of amphetamine in brain amine concentrations. The long term effects of PCA include decreases in brain serotonin (5-HT), tryptophan hydroxylase and changes in brain amines. The long term effects of PCA include increases in brain 5-hydroxyindoleacetic acid concentrations. Short term effects of PCA include increases extraneuronal 5-HT and various effects on central catecholamines. During the initial period, rats injected with PCA display a precoyced behavior which may not be altered by short term effects of PCA. The effects of PCA on the central nervous system involve in these effects.


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 is N,N-dimethyltryptamine (DMT) which has been the most vigorously pursued.

We have already reported dose response disruptive effects of DMT on rat shuttle-box 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 rats were trained to a high stable conditioned avoidance response (CAR) baseline rate (450 CARS). Pretreatment 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 (2.5 mg/kg, n=8) reduced punished responding. (DMT) (100 mg/kg, n=10); 1,2,5,6-tetrahydro-3-(N,N-diethylamino)- (THD) (THD) (1 mg/kg, n=10) postulated to occupy the DMT receptor (20 mg/kg, n=10). Cross-tolerance is the phenomenon which 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 which may be operating via different mechanisms in the brain. A learning interpretation of cross-tolerance can explain the phenomenon by positing compensatory changes in learning, such as altered physiological/psychological "state" as the mechanism underlying cross-tolerance. A 3-group design (intoxicated practice vs. drug exposure vs. saline controls) was used to study the effect of DMT (n=10 each). The results indicate that chronic, but not acute, neuroleptic pretreatment is effective in preventing disruptive effects of DMT may be due to the time dependent effects of neuroleptics on dopamine turnover and the therapeutic latency of these agents.


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 hr were allowed 200 unpuhished licks in the test apparatus to decrease intersubject variability in the subsequent drug test. After 48 hours of deprivation, the rats were allowed to drink for 3 min., but after every 20 licks, licking was punished for 2 sec. by electric shock through the water spout. ETOH (0.5-1.5 g/kg, 10 ml/w/v, ip) increased punished drinking, although not as strongly as CO2 (4-27 mg/kg, ip). Punished drinking was unchangeable by 2 g/kg and decreased by 2.5 g/kg ETOH. In contrast to our previous report, the effect of ETOH was not increased by 2.5 g/kg ETOH. The anti-conflict action of ETOH (1 or 2 g/kg) was maximal 15 to 60 min. post injection but could also be observed 2 or 20 hours after TRH administration.

Intracerebral injection of TRH (100 µg/rat) also increased punished responding, suggesting that the locus of this action of TRH may be in the hypothalamus. (DMT) (4-27 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 with any other type of tolerance. 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 (Miller et al., Arch. Gen. Psychiatry, 28:1971; 1971). (Supported by NIMH, AA-0334, MH-00013, MH-05363 and AA-05047.)


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 that may be operating via different mechanisms. A learning interpretation of cross-tolerance can explain the phenomenon by positing compensatory changes in learning, such as altered physiological/psychological "state" as the mechanism underlying cross-tolerance. A 3-group design (intoxicated practice vs. drug exposure vs. saline control) was used to study the effect of DMT (n=10 each). The results indicate that chronic, but not acute, neuroleptic pretreatment is effective in preventing disruptive effects of DMT may be due to the time dependent effects of neuroleptics on dopamine turnover and the therapeutic latency of these agents.

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 (15mg/kg) induced activity; 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 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 impacations 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.


Recent studies suggest that levels of urinary MHPG may predict the occurrence 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 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 unipolar patients, the occurrence 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 treatment 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 = .57, n = 14, p < .001).

Furthermore, patients who developed severe and orolmented 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 low MHPG patients treated with IMI, a finding consistent with data from unipolar patients. The theoretical implications of these findings revolve around the postulated noradrenergic involvement in the switch process from depression into mania will be discussed.
RECEPTORS
MULTIPLE HIGH-AFFINITY BINDING SITES FOR BUTYROPHENONES IN RAT STRIATUM. Anne C. Anders and Michael E. Maguire, Dept. of Psychiatry and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

3H-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 approximatively 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 µM. Specific binding determined by physiologically relevant concentrations of putative dopaminergic agonists and antagonists, serotonergic agonists, and α-adrenergic agonists and antagonists. α-Adrenergic agents, γ-aminobutyric acid, and muscarinic agents had no effect on 3H-SP binding at concentrations of 1 mM or less. Because Scatchard analysis of (+)-butaclamol competition against 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 (Kd = 100 µM) was not effectively competed by any of these agents. Dopamine, phenotolamine, and serotonin all competed effectively for a single site (Kd = 1.5-2 µM, n = 300-400 fmol/mg) at concentrations above 10 µM. However, even at 3 µM 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 (Kd = 0.1 µM, n = 12, 50 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 antagonist of a putative 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.

DISPERAL AND REFORMATION OF ACETYLCHOLINE RECEPTOR CLUSTERS IN CULTURED RAT MYOTUBES. Robert J. Bloch. Dept. of Neurobiology, The Salk Institute, La Jolla, CA 92037.

Acetylcholine receptor (AChR) clusters in the plasmalemma of rat myotubes are lost upon treatment of cultures with inhibitors of energy metabolism. This test 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.

EFFECT OF ELECTROCONVULSIVE SHOCK TREATMENT ON MONOAMINERGIC RECEPTOR BINDING SITES IN RAT BRAIN. D. A. Bergstrom, F. Ladarola, K. J. Killar. 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 α-adrenergic binding sites in rat cerebral cortex without affecting serotonin or dopamine binding sites.

Sulzer and his associates have reported that daily administration of electroconvulsive shock treatment (ECT) to rats for 8 days results in a decrease of the norepinephrine-sensitive cyclic AMP generating system in brain. We have now 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 days. 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 α-adrenergic receptor binding sites were measured with [3H]-dihydralaprominol, the α-adrenergic agonist prazosin, and the serotonin receptor with [3H]-serotonin. The data were analyzed with Scatchard plots.

It was found no difference in the kinetic binding properties of the α-adrenergic, α-adrenergic, or serotonergic binding sites in rat brain cortex after a 1 or 2 day electroconvulsive shock regimen. However, with a consecutive 2 day electroconvulsive shock regimen 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 dopamine.

Antidepressant drugs and ECT produce a rapid increase in the availability of norepinephrine to post-synaptic receptors. The present findings with rats and ECT and with chronic antidepressant drug administration suggest that the delayed onset of these antidepressant treatments may be related to an adaptation of the α-adrenergic receptor binding sites to an increased availability of norepinephrine.

(Supported by US PHS NS 12566 and NASA NCA2R258701)
1628 NICOTINE CAUSES AN INCREASE IN SENSITIVITY TO MECHANICAL STIMULATION IN A CONTRACTILE PROTOZOA. Peter A. Boxer* and David C. Wood. Psychobiology Program, Dept. of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

A supraphrenial 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 and other workers (56C, 151, 1977) have shown that the organisms sensitivity to mechanical stimuli can be markedly reduced by addition of 3-600mM d-tubocurarine chloride (d-T). Our recent nicotinic cholinergic antagonist studies show that the effect of d-T is specific to mechanical stimulation; d-T must bind to the site involved in mechanical stimulus transduction. Based on this specificity a [14C]-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 nicotine agonists. Upon addition of nicotine (0.5 mM) to the medium there is an initial decrease in the probability of contraction to mechanical stimuli followed by a 1-3 hr significant increase in the probability of contraction in these 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 deactivation of the contractile response, while the increase in sensitivity is specific to mechanical stimuli.

Nicotine also appears to compete with d-TC at the mechanical transduction site. Incubation in nicotine (0.5mM) for 1 to 3 hrs. causes a 90% inhibition in the amount of bound TC-d-TC while shorter incubations had little effect. Therefore there is a correlation between the ability of nicotine to inhibit the binding of [14C]-d-TC and its ability to increase the sensitivity of the organism to mechanical stimuli.

Other cholinergic agonists (1.0mM Dimethylphenylpiperazinium (DMPP), 1.0mM Carbamylcholine (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 1 hr. later. These changes produced smaller effects on the binding of [14C]-d-TC than nicotine. The finding that nicotine and other cholinergic agonists affect mechanical stimulus transduction by acting on a curasiform 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.


After culturing ganglia, rather than dissociated sympathetic ganglia whole, rather than as dissociated sympathetic ganglia, 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 receptor complexes are incorporated into the ganglion than the axons and that the rate of incorporation of receptors, expressed as per cent of the surface area 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.


We have previously demonstrated that α-bungarotoxin binding is selective to cholinergic neurons that this binding is saturable (100nM) 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 (PMAS 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 NaCl or 1M EDTA but is readily extracted by non-ionic detergents (Triton X-100, Emulphogene BC720). α-Bungarotoxin receptor complex is normally dissociated with a half-life of 3.5 hrs but after cross-linking, a-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 receptor).

Neurons grown in medium containing "heavy" 3H, 125I, 131I-substituted amino acids incorporate these heavy amino acids into α-bungarotoxin receptors during protein synthesis, and the resulting heavy receptors can be separated by light receptors by velocity centrifugation in 25-40% sucrose-deuterium oxide gradients. We have applied this technique to chick sympathetic neurons from 2 day old cultures and have been able to separate the α-bungarotoxin receptor to obtain information about the kinetics and the localization of membrane synthesis in growing neurons. Of the kinetic features 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. This 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.

As ganglia are 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 receptor complexes are incorporated into the ganglion than the axons and that the rate of incorporation of receptors, expressed as per cent of the surface area 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.

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 (heaver 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 3H-flunitrazepam binding in the purified astrocytic and neuronal fractions show that both fractions display saturable binding with 

\[
K_d \approx 30 \text{ nM}, \quad \text{IC}_{50} \approx 5,000, 32, 28 \quad \text{and} \quad 22,000 \text{ nM respectively.}
\]

50'-hydroxydopamine. None of these lesions reduce BZR binding in the neuronal types except granule cells by intracerebellar kainic acid administration, mutation leading to loss of granule cells (heaver 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 3H-flunitrazepam binding in the purified astrocytic and neuronal fractions show that both fractions display saturable binding with 

\[
K_d \approx 30 \text{ nM}, \quad \text{IC}_{50} \approx 5,000, 32, 28 \quad \text{and} \quad 22,000 \text{ nM respectively.}
\]


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 (heaver 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 3H-flunitrazepam binding in the purified astrocytic and neuronal fractions show that both fractions display saturable binding with 

\[
K_d \approx 30 \text{ nM}, \quad \text{IC}_{50} \approx 5,000, 32, 28 \quad \text{and} \quad 22,000 \text{ nM respectively.}
\]


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 (heaver 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 3H-flunitrazepam binding in the purified astrocytic and neuronal fractions show that both fractions display saturable binding with 

\[
K_d \approx 30 \text{ nM}, \quad \text{IC}_{50} \approx 5,000, 32, 28 \quad \text{and} \quad 22,000 \text{ nM respectively.}
\]
The "opiate" receptor was first identified using a radio-labeled 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. Pharmacol. 41: 247-248, 1977). The tritiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977). The titiated ligands used were: agonists morphine, dihydromorphine; antagonists: naloxone and naloxone; peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6. The binding assay was performed as described previously (Prog. Neuro-Psychopharmacol. 1: 259-264, 1977).
\[ \text{3H-Spiroperidol binds to two receptor sites in both rat frontal cortex and corpus striatum. J. Z. Fields, N.W. Pedigo*, T. D. Reisine, and H. I. Yamaura. Dept. of Pharmacology, Univ. of Arizona Health Sciences Center, Tucson, Arizona 85724.} \]

Spiroperidol is both a potent antipsychotic drug and a potent dopamine antagonist. \[ \text{3H-Spiroperidol (3H-SP) (26.4 Ci/mmol) binds to receptor sites in the frontal cortex (FC) and corpus striatum (CS) of rat brain. In the CS, these 3H-SP binding sites have been called dopaminergic because pharmacologically active dopamine agonists and antagonists are the most potent inhibitors of 3H-SP binding. The dissociation constant (K_d) in this area, determined by kinetic experiments using the ratio of the rate constant (k) of 1640 and 1642, may be non-dopaminergic in nature, as recently suggested by Leysen et al. (2012). Alternatively, either of these binding sites may represent a low-affinity binding site in CS is the same as the low-affinity binding site in rat striatal tissue. The high-affinity binding site in FC is revealed only at the lower concentrations of 3H-SP used in saturation studies (less than 15 pM). Scatchard analyses of 3H-Sp binding at ligand concentrations of 20 pM to 5000 pM indicate a second, low-affinity binding site with a K_d of approximately 340 pM at an [R] of 5 to 9 pM. The density of low-affinity binding sites in the rat FC (approximately 330 pM at an [R]t ≈3 pM) gives the "true" K_d. In rat striatal tissue, the extrapolated "true" K_d is approximately 10 pM, which is in good agreement with the data from our kinetic experiments.} \]

\[ \text{3H-Spiroperidol binding in rat FC is complicated by the existence of two distinct receptor sites. The high-affinity 3H-Sp binding site in the FC has a K_d similar to the K_d observed in the CS (20 pM). However, the density of high affinity sites in the FC (>2.5 pmol/g tissue) is 16 times less than in the CS (40 pmol/g tissue). The high-affinity binding site in FC is revealed only at the lower concentrations of 3H-SP used in saturation studies (less than 15 pM). Scatchard analyses of 3H-Sp binding at ligand concentrations of 20 pM to 5000 pM indicate a second, low-affinity binding site with a K_d of approximately 340 pM at an [R] of 5 to 9 pM. The density of low-affinity binding sites in the rat FC (18 pmol/g tissue) is approximately 7 times greater than the density of high-affinity sites.} \]

In light of these data, saturation studies were done in rat CNS using high concentrations of 3H-SP (up to 5000 pM). These experiments indicated that a low-affinity binding site is present in rat CNS but at a relatively low density (<14 pmol/g tissue). The K_d 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] of 3 pM). Thus, this K_d appears to bind to the FC and CS. The high-affinity binding site shows similar affinity 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.

\[ \text{The delayed increase in TH activity following hypoxia with the observed induction of TH reported by Hanbauer et al. How­} \]

ever, the basal TH level which we find for rat carotid body is more than 10 times higher than the TH level of the rat. It is difficult to reconcile such a large discrepancy to differences in the assay method (Nagatsu et al., 1976; Nagatsu et al., 1977; Nagatsu et al., 1978). 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.)

\[ \text{A COMPARATIVE STUDY OF THE EFFECTS OF HYPOXIA ON TYROSINE HYDROXYLASE ACTIVITY IN THE CAROTID BODY OF RAT, RABBIT AND CAT.} \]


The activity of tyrosine hydroxylase (TH) is known to be stimu­}

lated by hypoxia. Costa and Cohen (1977) demonstrated an induction of TH in rat carotid body at 3 and 2 days following exposure of the animals to 1 hr. to 5% O_2 for 30 min and 30 min exposure to 20% oxygen. However, the observed induction of TH reported by Hanbauer et al. How­}

ever, the basal TH level which we find for rat carotid body is more than 10 times higher than the TH level of the rat. It is difficult to reconcile such a large discrepancy to differences in the assay method (Nagatsu et al., 1976; Nagatsu et al., 1977; Nagatsu et al., 1978). 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.)

\[ \text{ISOELECTRIC FOCUSING VARIANTS OF THE NICOTINIC ACETYL­} \]

\[ \text{CHOLINE RECEPTOR FROM DROSOPHILA MELANOGASTER. Linda M. Hall, Thomas H. Hudson, Paul W. von Borstel, Barbara C. Osmund and Sydney D. Houlton.} \]

\[ \text{Biology Department 16-711, M.I.T., Cambridge, MA. 02139.} \]

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 functions and in the acetycholine receptor are members of the nervous system. We are particu­}

larly interested in isolating mutants affecting the acetylcholine receptor, because of the key role that this component plays in synaptic functions and in the production of acetylcholine. 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 the nervous system. We are particu­}

larly interested in isolating mutants affecting the acetylcholine receptor, because of the key role that this component plays in synaptic functions and in the production of acetylcholine. 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 the nervous system. We are particu­}

larly interested in isolating mutants affecting the acetylcholine receptor, because of the key role that this component plays in synaptic functions and in the production of acetylcholine. 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 the nervous system. We are particu­
CULTURED HUMAN ASTROCYTOMA CELLS: DIFFERENTIAL EXPRESSION OF COMPONENTS DURING CELL GROWTH.
T.K. Harden, S.I. Foster*, and J.P. Perkins*, Dept. of Pharmacology, Univ. of N.C. Med. Ctr., Chapel Hill, N.C. 27514

Cultured human astrocytoma cells (132 INI) respond to iso-
propranolol (150 nM) with marked increases in cyclic AMP levels and
release of cAMP into the medium at sites in intracellular CAMP levels. The amount of cAMP that accumulates in intact cells in response to maximally effective concentrations of isoproterenol (100 nM) and PGE1 (100 nM) was again increased
by time of subculture of cells. ISO-stimulated CAMP accumula
tion increases 3-4 fold during the first 2 days after subcul
ture, then subsequently declines (10% of ISO responsiveness
after 8 days of culture. In contrast, PGE1 responsiveness
gradually increases by approximately two-fold over an 8 day period. Such changes in the expression of the components of the hormone receptor/adenylyl cyclase system was carried out to
determine the mechanism(s) responsible for this differential expres
sion of astrocytoma cell responsiveness to hormones. The
changes in intact cell responsiveness were reflected by similar
alterations in hormone stimulated adenylyl 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. PGE1-stimulated adenylyl cyclase activity gradually
increased by 1.5-3.0 fold during 8 days of culture. A small in
crease (1.2-1.5 fold) was observed in basal and NaF stimulated
enzyme activity during the first 48 hr after subculture. The
role of (n)-adrenergic receptors in the observed changes in ISO
responsiveness was examined using (-)-hydroxybenzylpindolol
(SERP) as a specific receptor ligand. The affinity (Ki) of SERP for ISO in unstimulated cells is 3.5-5 times lower than the receptor density observed after 6 hr of culture. During the following 6 days receptor density decreased, reaching a level that was 24-30% of the original level. When cells were plated at high density (> 2x10^6 cells/cm^2), no increase in ISO-responsiveness and (n)-adrenergic receptor density occurred
during the period of subculture. In addition, there was a significant
increase in ISO responsiveness and a decrease in (n)-receptor density in this regulation of the components of the CAMP system is under further investigation. Supported by HL 22490 and
GM 25163.

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
RECEPTORS OF INVERTEBRATES.

SOCIETY FOR NEUROSCIENCE
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.


The ability of cholinergic agonists to displace "H-Na-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-NEM 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 DTNB-treated nAChR is markedly altered, IC-50 values being <2 µM for coincubation. However, DTNB-reduced nAChR is responsive to pretreatment with ACh, as IC-50 values are >50 µM for the preincubation case. Furthermore, a log 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 to 10 nM α-Bgt is increased on nAChR or the affinity state of 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 or the 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 NINDS postdoctoral fellowship (RJL).
with dopaminergic dendrites in the SN, these data suggest that GAD than with striatal TH. The localization of DA receptors in activity in the striatum. There was no correlation of QNB binding—

destroy neurons in the striatum without injuring afferent dopa-

ence of nerve ending. Among the neurons destroyed are 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 recep-

tors in the striatum and DA receptors in the SN by standard bind-

ing assays with [3H]QNB, [125I]a-bungarotoxin (α-Btx) and [3H]

piperidin 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 affer-

ents [3]. Since dopaminergic nerve endings connect with cholinergic dendrites in the striatum, and GABAergic nerve endings connect with dopaminergic ones, the above data suggest that dopamine and acetylcholine may be released from dendrites to act on receptors on axonal endings. They support morphological stud-

ies of Gotts, McGeer, and McGeer, Dendroaxonic Neurotransmission II: Evidence of nerve ending function in postynaptic membrane vesicles from Torpedo californica. Specifically, the phospholipase A inhibition shows the following properties: 1) it occurs at concentra-

tions of 50 times lower than those required for the induction of inhibi-

tion by α-neurotoxin; 2) the phospholipase A has no effect on the binding properties of the receptor; 3) the inhibition is abol-

ished by removal of calcium ions; and 4) some phospholipid hy-

drolases accompanies inhibition. Introduction of free fatty acids or lysophosphatidylcholine into the membranes also results in inhibition of receptor activation, although much higher concen-

trations than those generated in situ are required. Lysophos-

phatidylethanolamine does not inhibit receptor function.

The inhibition by phospholipase A2 or by fatty acids and lys-

ophosphatidylcholine can be completely reversed by treatment of the membranes with bovine serum albumin. Inhibition and its reverse can be selectively correlated with the uptake and removal of fatty acids, using both radioactive and spin-labeled fatty acids.

It is suggested that phospholipase A2 acts enzymatically to produce fatty acids and some lyso-derivatives that effectively uncouple ligand binding from ion permeability in the receptor containing membrane vesicles.

CHARACTERIZATION OF STRIATAL [3H]APOMORPHINE AND [3H]SPIRO-

PERIDOL BINDING AND DOPAMINE SENSITIVE ADENYLAZE CYCLASE

ACTIVITY UTILIZING PHOSPHOLIPASE A2 ALKALOIDS. L.K. Moyerson, B. George*, M. Abel*, H. Phillips*, V.E. Days and Y.C. Clement-Cormier. VA Hospital, Houston, Texas 77211, Baylor College of Medicine, Houston, Texas 77030. The University of Texas Medical School at Houston, Houston, Texas 77025.

Tetrahydroprotoberberine alkaloids contain a nitrogen atom in a fixed position (cisgauche) to the catechol nucleus. These compounds inherently possess the ability to incoquinoline moieties forming part of their molecular geometry. The recent availability of several positional and optical isomers in various states of phenolic methylation and nitrogen quatemarization made it possible to further characterize the geometric, stereospecific and topographic requirements for agonist and antagonist radioligand binding to the dopaminergic receptor. The optical isomers of 2,3,9,10- and 2,3,10,11-tetrahydroxyprotoberine (THPB) and selective mono-O-methylation of 2,3,10,11-THPB were tested for their ability to displace 5nM [3H]apomorphine and 0.2nM [3H]spiroperidol and to inhibit adenylyl cyclase activity in the presence of 100 µM dopamine. The results of these studies are shown in the table below:

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>[3H]-APO Specific Binding</th>
<th>[3H]-SPIRO Specific Binding</th>
<th>DA-Cyclase IC50 (nM)</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-2,3,9,10-THPB</td>
<td>60</td>
<td>500</td>
<td>3000</td>
<td>3000</td>
</tr>
<tr>
<td>(+)-2,3,10,11-THPB</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>10000</td>
</tr>
<tr>
<td>(+)-1,6,10,11-THPB</td>
<td>150</td>
<td>450</td>
<td>59000</td>
<td>59000</td>
</tr>
<tr>
<td>(+)-1,6,8,9,10,11-THPB</td>
<td>150</td>
<td>450</td>
<td>59000</td>
<td>59000</td>
</tr>
<tr>
<td>(+)-2,3,10-THPB</td>
<td>2000</td>
<td>1200</td>
<td>15000</td>
<td>15000</td>
</tr>
</tbody>
</table>

These data indicate that the (+)- isomer of both THPB was more potent than the (R)-isoemer in displacing labelled apomorphine and spiroperidol from their respective binding sites. Selective mono-O-methylation altered the potency of these compounds to displace antagonists or agonist labelled re-

Two sets of data are presented below: 1) the inhibition of non-myelinated fibers and their origin in sympathetic ganglia, and 2) the inhibition of postganglionic fibers arising from cell bodies in the superior cervical ganglion. The larger non-myelinated fibers appearing individually in the Schwann cell are the sensory distal processes of petrosal ganglion cells. Preliminary studies indicate that these fibers degenerate after ganglionectomy, while the small diameter non-myelinated fibers degenerate after section of the spinal cord or fibers having their origin in sympathetic ganglia below the superior cervical ganglion are present in the carotid sinus nerve.

SOCIETY FOR NEUROSCIENCE

FIBER SIZE CONTENT AND ORIGIN IN THE RAT CAROTID SINUS NERVE.


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 ganglion-

ectomy (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 80-100 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 2µm. The 5-10 larger fibers are about 4-5µm 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 1-1.5µm in diameter. Smaller non-myelinated fibers (0.3-0.5µm 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 share a Schwann cell, and in origin are postganglionic fibers arising from cell bodies in the superior cervical ganglion. The larger non-myelinated fibers appearing individually in the Schwann cell are the sensory distal processes of petrosal ganglion cells. Preliminary studies indicate that these fibers degenerate after ganglionectomy, while the small diameter non-myelinated 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 2µm in diameter are still present, scattered throughout the nerve and surviving the surgical procedures; the origin of these fibers remains obscure. Section of pregangli-

onic fibers to the superior cervical ganglion results in no degeneration of non-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 RO8558.)
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 (AChR) 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 are found in 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 (Salvattori & 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 10 days.

We now report a thorough study of BTX binding sites in rat brain during development utilizing a biochemical assay for 125I-BTX. In addition to large regions (cortex, forebrain, brainstem, cerebellum), several areas throughout the brain (e.g., olfactory bulbs, inferior colliculus, superior colliculus, raphe, reticular formation) were also investigated on days 1-5 postnatal.

In the cortical/forebrain brain levels of BTX binding sites was high (1.5 pM BTX sites/g wt.; .04 pM/mg pro.). In general the development of BTX binding sites follows a caudal-to-rostral pattern and appears to increase gradually postnatally to adult levels 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 dissociation constants of BTX binding by cholinergic ligands throughout development, suggesting that there is no change in the conformations 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 these 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.

ACETYLCOLINE (AcCh) AND LOCAL ANESTHETIC BINDING PROPERTIES OF TORPEDO CAL, NICOTINIC POST-SYNAPTIC MEMBRANES AFTER REMOVAL OF NON-RECEPTOR PEPTIDES


A comparison of the peptide composition of post-synaptic membranes isolated from Torpedo electric tissue with that of the nicotinic cholinergic receptor (AcCh) isolated in detergent solution from that tissue revealed a major difference. Resides in a 43,000 dalton peptide (43K) present in the membranes, but absent in the isolated AcCh (Sobel et al., Eur. J. Biochem. Bj 215, 177 (1977)). The 43K protein is also 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 AcCh receptor in the synaptic membranes we have 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 dodecylsulphydryl/polyacrylamide gel electrophoresis of the peptide composition of the 43K-depleted membrane established peptide components of 40, 50, and 60,000 daltons, peptides characteristic of isolated AcCh. The 43K-depleted membranes contained 40% of the protein and 80-90% of the initial [3H]-AcCh binding sites. Functional integrity of the 43K-depleted membrane was measured by: (1) an analysis of the kinetics of binding of [3H]-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 [14C]-trimethoprim. 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 [3H]-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 10M dimethylsulfoxide the AcCh binding attained equilibrium in less than 1 sec. Furthermore, both 43K depleted and control membranes, the [14C]-trimethoprim was bound with a dissociation constant Kg=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 NDAA fellowship to EKX.


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 of the goldfish brain (21B (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 125I-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 10^8 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 KI's for cholinergic ligands: d-tubocurarine=10.7 nM, nicotine=36.3 nM; carbamylcholine=1.3 µM; hexamethonium=11.2 µM; and atropine=0.10 nM. 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. The toxin-receptor complex is readily resolved by gel filtration on a Sephadex G-100 column, assuming a Stokes radius of 68 ± 1.2A and a diffusion constant of 2.4 x 10^-7 cm^2/sec for the toxin-receptor-detergent complex. Sedimentation velocity experiments in sucrose-H2O and sucrose-H2O 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 complex is approximately 700,000 daltons, assuming a Stokes radius of 68±1.2A and a sedimentation constant of 11.3 S. 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. The toxin-receptor complex is readily resolved by gel filtration on a Sephadex G-100 column, assuming a Stokes radius of 68 ± 1.2A and a diffusion constant of 2.4 x 10^-7 cm^2/sec for the toxin-receptor-detergent complex. Sedimentation velocity experiments in sucrose-H2O and sucrose-H2O 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 complex is approximately 700,000 daltons, assuming a Stokes radius of 68±1.2A and a diffusion constant of 2.4 x 10^-7 cm^2/sec for the toxin-receptor-detergent complex.
MECHANICAL PROPERTIES OF THE INTRASFAL MUSCLE OF MAMMALIAN MUSCLE SPINDLES. E. E. Poppele, W. R. Kennedy, and R. 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 (<100 µm) were applied to isolated muscle spindle 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 stretched 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 into receptor potentials.

(Supported by NSF grant #76-10791 and USPHS grant NS 109690-04.)

PRE- AND POSTSYNAPTIC LOCALIZATION OF NEUROTTRANSMITTER RECEPTORS IN THE RAT CORPUS STRIATUM. T. D. Reisine, J. L. Nagy, B. C. Pitliger and H. Y. 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 (3H-spiperidol), muscarinic cholinergic (3H-QNB, benzilate, DMB) and beta-adrenergic (3H-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 30% decrease in the Bmax of 3H-spiperidol binding with no alterations in 3H-QNB binding. There was, however, a significant 30% decrease in the Bmax of 3H-DHA binding within the CS of these treated rats. There was also a significant 40% decrease in 3H-spiperidol 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 3H-spiperidol binding in the treated rats indicates the existence of DA receptors on DA perikarya or dendrites. Supported by USPHS grants and RCD.

DISSOCIATION BETWEEN THE PRESYNAPTIC DOPAMINE SENSITIVE ADENYLATE CYCLASE CYCLE AND 3H-SPERPERONE BINDING SITES IN RAT SUBSTANTIA NIGRA. Marjka Quik, Piers C. Emerson, Eileen Joyce and Leslie L. Tversen


Seven or 14 days after lesions which destroyed various components of the striato-nigral system, 3H-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 3H-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 GAD unaffected, resulted in a 40% decrease in 3H-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%). These results suggest that within the substantia nigra the DA receptor sites defined by using 3H-spiperone are located mainly postsynaptically, while the DA-sensitive adenylate cyclase is located pre-synaptically.


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, interspinals, transversospinals 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 perivertebal 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 paciniform 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 courses within loose connective tissue particularly near blood vessels. However nerves were not observed in highly structured tendons or synovial linings. The obvious receptor of the neck were thus not joint receptors but muscle receptors in perivertebral muscles around vertebral articulations.

Supported by M.R.C. of Canada
The presence of brain-specific benzodiazepine receptors is now established and there is a significant correlation between the affinities of various benzodiazepines and the anxiety-eliciting 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.

In every brain region examined, the MNR rats showed higher specific benzodiazepine binding than the emotional MK 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 cerebral 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.

Thus, 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 findings of a difference in benzodiazepine receptor binding between 2 strains representing extremes of emotionality may provide a biological basis for an understanding of emotional behaviour based on alterations in the benzodiazepine receptor between 2 strains of rats representing the extremes of emotionality.

Measurement of specific 3H-diazepam binding (at 2nM 3H-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 MNR strain showed higher specific benzodiazepine binding than the emotional MK strain 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 cerebral 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 strain is due to an increase in the number of binding sites rather than a change in the affinity of the receptor for the ligand.

A preliminary step in designing effective experimental procedures for isolating and characterizing the neuronal benzodiazepine receptors has been the study of the effects of various physical, chemical and enzymatic treatments on the particulate binding sites for [3H]diazepam, [3H]flunitrazepam, and [3H]Ro16-2903.

Membranes were prepared from rat forebrain by a modification of a modified flotation-sedimentation procedure (Jones and Matus, Biochem. Biophys. Acta 388:276, 1974) using a fixed angle rotor. This procedure yields a synaptosomal membrane enriched fraction as judged by electron micro-scopy and enzymatic activity (5-7 fold enrichment of Na+ K+ATPase). The specific activity of a-BeTX and GMB 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) (10-4-10-1M) had little effect on GMB binding. β-Mercaptoethanol had only a slight inhibitory effect on α-BeTX 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 10-5M. The alkyating agent N-ethylmaleimide shows 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 phospholipase is due to an increase in the number of binding sites.

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 effector nerve carrying such information to the brain is the vagus, since its section 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 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, chloral chloride, mannitol, or sucrose was infused simultaneously such that the ionic/osmotic challenge 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 field tests on hepatic membranes with amounts of horseradish peroxidase were lontopherosed 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 DR and NS 10928 to L.L.B.)

High affinity dimethyltryptamine binding to rat brain membranes. Helen Rosengarten* and Arnold J. Friedhoff, Millhauser Labs, Dept. Psychiatry, N.Y. Medical Center, New York, NY 10016.

Postsynaptic membranes of rat brain were found to bind dimethyltryptamine. Binding of 3H-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 3H-DMT to membranes of rat brain.
The use of fluoxetine in the binding assay for serotonin receptors


Because of the possibility of abnormalities in central serotoninergic dysfunction, 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, (Nol. Pharmacol. 7, 1976). It was found that when crude mitochondrial fractions prepared from rat cerebellar cortex were incubated for 15 min. at 30° with concentrations of [3H]-serotonin (3H-SHT) up to 40nM, a population 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 (Kd) and maximum specific binding capacity (Vmax) of the higher affinity site were 7.0 ± 0.25nM and 170 ± 14fmole/mg protein respectively, (N=4). For the lower affinity site, the Kd was 30 ± 4.2nM and the Vmax was 453 ± 25fmole/mg protein, (N=4).

The effect of fluoxetine, a specific serotonin uptake inhibitor, on 3H-SHT binding was examined to investigate whether the lower affinity site was some form of a binding site for serotonin uptake inhibition. Fluoxetine in concentrations up to 0.5µM, produced no significant difference 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 3H-SHT to a lower affinity population of binding sites, the characteristics of which are under investigation. The binding sites for 3H-SHT measured in the presence of fluoxetine exhibit the same affinity characteristic of serotonin receptors as determined by the order of potency of the following drugs in displacing 3H-SHT: serotonin > 5-LD-5-methoxytryptamine > tryptamine > methysergide > serotonin > 5-hydroxytryptamine, atropine > dopamine, norepinephrine, isoproterenol. (Supported by Research Funds from the Veterans Administration, NIH Grant MH29209, and USPHS GM 07302).


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 poles of the cerebellar hemispheres were removed from humans of various ages 6-16 hrs after death. Neurotransmitter-dependent cyclic AMP accumulation was measured in tissue slices from a lower dihydroalprenolol binding, adenylate cyclase assay. The effects of age and their second differences were computed to localize the densities of the sources and sinks of synaptic current. 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).

Vertebrate photoreceptors respond to light with membrane hyperpolarization. A striking characteristic of these responses is that for bright flashes (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)
SELECTIVE BLOCKADE OF HYPOTHALAMIC CHOLINERGIC PATHWAYS BY
ANTIBODY TO GM1 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(αGS) have shown EEG abnormalities and in-
hibition of a passive avoidance task to be induced in rats follo-
wing intracranial administration of αGS. (Karpjac et al, Science
strated that this neuroactivity was eliminated by absorption with GM1
ganglioside. We have found that focal intrahypothalamic injection
of αGS (administered 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 (αRB) antibody (Williams
and Schupf, Science 196:328, 1977). A discriminating assay system,
pharmacological induction of food and water intake, demonstrated
that the activity of αGS appeared to be selective for inhibitory
hypothalamic neurons mediating adrenergic-cholinergic antagonism.
This test was employed to determine the functional activity of
αGS.

Rats were prepared with cannulae in the perifornical hypothal-
amus, 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
carbachol (CC) will elicit excessive drinking. Treatment
with αGS (2 ng in 1 μl) 6 hours before drug stimulation depressed
drinking in CC-stimulated rats but produced no change in aggre-
tative behavior in either NE-stimulated or saline control rats.
The activity of the antibody was eliminated by absorption with GM1
ganglioside. These results suggest that the neuroactivity of αGS
in this brain region is best defined by blockade of cholinergic
neurons. This discrete activity contrasts with that reported for
αRB.

While GM1 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.

PRE-SYNAPTIC Dopamine receptors Labeled by 3H-apomorphine.
P. Seeman and J. Tedesco, Department of Pharmacology,
University of Toronto, Toronto, Canada MSS 1A8

Previous work has shown that 3H-apomorphine (at 3 nM) prefers
pre-synaptic dopamine receptors over post-synaptic dopamine re-
ceptors in the caudate, since 6-hydroxydopamine lesions of the
nigrostriatal fibers caused a 56% fall in the binding of 3H-
apomorphine, but a 22% rise in the binding of 3H-haloperidol.
Intrastriatal injections of kainic acid, moreover, had no effect
on the striatal binding of 3H-apomorphine but did lower
the binding of 3H-spiradoline, supporting the theory that 3H-apo-
morphine favours attachment to pre-synaptic dopamine sites, while
3H-spiradoline prefers post-synaptic dopamine receptors.

To test further the hypothesis that 3H-apomorphine (at 3 nM)
preference pre-synaptic dopamine receptors, we tested a number of
dopamine-mimetic compounds for their ability to compete with
3H-apomorphine binding; we then compared these competing concen-
trations with those which are known to act on peripheral pre-
synaptic dopamine receptors. The concentrations which 50%
inhibited the specific binding of 3H-apomorphine to calf caudate
homogenates are shown in the Fig. In correlation to the concen-
trations which 50% inhibit the cardioacceleration responses3-5.

The results are thus compatible with the theory that 3H-apo-
norphine (at 3 nM) may bind to high affinity pre-synaptic sites
for dopamine.

(Supported by NRC of Canada and the Ontario Mental Health
Foundation).


INGENUOCHIMICAL 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 immunologically
identical.

When antisera anti-SBP-CIP (or antisera anti-SBP-LSD) was
reacted with rat brain synaptosomal membranes, one immuno-
precipitin line was observed and it was immunologically
identical to the purified SBP from steer hypothalamus. Further-
more, both antibodies inhibited specific serotonin-binding to a
similar degree.

MUSCARINIC ACETYLCHOLINE RECEPTOR REGULATION IN CULTURES OF
EMBRYONIC CHICK BRAIN. Robert G. Siman* and William L. Klein*
(EMB: Virginia Carr). Dept. of Biological Sciences, North-
western 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
when prepared from 8 day old chick embryos and maintained for
7 days have about 100 fmol receptors/mg protein. These cul-
tures 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 recep-
tors which closely resembles the receptor occupancy profile
for carbachol. A saturating dose (10-4M carbachol) lowers
receptor density by 75%. A half-maximal reduction in receptor
density is induced by 10-7M carbachol. The regulation of
muscarinic ACh receptor mediated and requires receptor activa-
tion and not simple occupancy. Thus 10-7M oxotremorine induces
a receptor loss which is blocked by 10-7M 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 by 48 hours after agonist, 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 IMIDA
ZOLINE DRUGS ON THE PINEAL α-ADRENERGIC RECEPTOR-MEDIATED STIMU-
LATION 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 32p into phospholipids of the rat pineal gland. The most prominent stimulation occurs in phosphatidylinositol, while modest increases are seen in phosphatidic acid and phos-
phatidylglycerol. Phosphatidylcholine and phosphatidylethanol-
amine are not affected. This increased incorporation of 32p 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 norepinephrine, a non-adrenergic antagonist, has no effect. A variety of α-agonists also enhance 32P 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 prontpyrine inhibit completely the stimulation of 32p 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 Kd for amitriptyline of about 1 μM. Incubations of pineal glands with 1.5 μM tricyclic antidepressants plus 5 μM NE yield an upward shift of the order of inhibitory potency tertiary amine > secondary amine > tertiary amine. This is not seen in other α-adrenergic systems. Among the imidazolines, clonidine, oxytocinamine, naphazoline, mepazolamine, and tolazoline antagonized the stimulation caused by 10 μM NE. Clonidine also exhibits partial agonist activity. Although many of the tricyclic and imidazoline drugs have strong local anesthetic properties which in effect block the target membranes phospholipids in a characteristic manner, these effects were not observed at the concentrations used. Since some of the imidazolines have been reported to act as presynaptic α-adrenergics, their action inhibiting increased 32P 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 het-
erolytic 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 specificity allosteric agents have been used as potential affinity labels for the bond (Karlin, Fed. Proc. 32, 1973). Using sodium bisulfite and the frog neuromuscular junction and hatchetfish Müller fiber-giant fiber synapse, I have produced a heterolytic cleavage of a disulfide bond with, unlike the reduction, produces an increased response to acetyl-
choline. No change in tissue course or impulse frequency was produced. The affinity labels used following reducing agents are also active follow-
ing heterolytic cleavage indicating that the same disulfide bond is in-
volved. The heterolytic cleavage results in the addition of a strongly nuclophilic sulfonate to a site near the cholinergic binding site and in-
creased 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 and plate po-
tentials (meppe's) and a decrease in the quantal content. The presynaptic response to modification of this disulfide bond has not been investigated previously and so the reducing agents used decreased the cholinergic re-
sponses. As can be seen, the most likely hypothesis for the increased frequency (up to 100/seconds) 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 po-
tentials is seen. This system 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 pre-
synaptically on the meppe frequency. Both these presynaptic effects can be blocked by prior treatment with postsynaptic N-methylacetylcholine. 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 mediat-
ing 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 [125I]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 centrifuga-
tion 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 bind-
ing. Specific VIP binding was reversible and saturable (Kd = 8 μM/gm tissue). Brain membranes exhibited a high affinity for VIP (Kd = 3 μM) 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. Natriuretic and aprotinin preserved binding, presumably by competing with endogenous processes, while soybean trypsin inhibitor was not effective. Binding of VIP paralleled its immunohistochemical localisation, being enriched in cerebral cortex, hippocampus, hypothalamus, striatum and thalamus. Secretin and partial sequen-

tic binding of VIP competed with membranes with Kd's of about 100-1000 times that of VIP. This finding is similar to that obtained in binding studies of the VIP receptor found in pancreatic acinar cells (Christophel et al., J. Biol. Chem. 244, 2641, 1969). The high affinity of VIP for membranes 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. Dedesco and R. Seeman. Pharmacology Department, University of Toronto, Toronto, Canada.

We have previously reported that 3H-apomorphine receptors in calf striatum crude homogenates (Seeman et al., J.N.A.S. 23, 4354, 1976; Seeman et al., Fed. Proc. 37, 130, 1978) are identical in all respects to high affinity 3H-dopamine receptors. In the current study we have determined that the lone electron pair of the tertiary amine nitrogen of apomorphine is necessary for the receptor binding. Dopamine itself has an IC50 of 2 nM when competing for binding to 3H-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- and N,N-dipropyl-dopamine have IC50 values of 10.5, 55, 74 and 625 nM, respectively. The secondary amine analogues N-methyl-, N-ethyl- and N-propyl-dopamine have IC50 values respectively of 2.5, 25 and 235 nM. In contrast the quaternary amine analogue N,N,N-trimethyl-dopamine has a very low affinity (IC50 = 1200 nM). This compound lacks a nitrogen molecule electron pair. Similarly, while R-(-)-apomorphine and K(-)-apomorphine have IC50 values of 3.8 and 4 nM, the quaternary amine analogue N-phenyl-apomorphine has an IC50 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 (Bellau et al., J. Med. Chem. 12(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.)
1682 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- and norepinephrinergic (3H-DHEC) in calf caudate homogenate. It is known that 3H-DHEC can selectively label to dopamine receptors. In the presence of 5 nM aspinepin, which serves to block dopamine receptors, the specific binding of 3H-DHEC was inhibited by 90% at 30 nM (-)-noradrenaline (Kd value is 260 nM (-)-norepinephrine, and 1000 nM dopamine, indicating that 3H-DHEC is selectively blocking to dopamine autoreceptors under these conditions. These numbers agree with those for 3H-WB-4101, published by others.

An important question, however, is that these inhibitory concentrations for 3H-DHEC do not match those which inhibit the binding of 3H-clonidine or 3H-norepinephrine. A simple and straightforward resolution of this dilemma would be to propose that 3H-DHEC and 3H-WB-4101 label the post-synaptic alpha-receptors, while 3H-clonidine and 3H-norepinephrine label the pre-synaptic autoreceptors. This suggestion is compatible with the data shown below indicating that the stimulated release of 3H-norepinephrine and the specific binding of 3H-clonidine are both inhibited by almost identical concentrations (or Kd values) of the drugs listed.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (nM)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>(-)-noradrenaline</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>50</td>
<td>37</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>100</td>
<td>102</td>
</tr>
</tbody>
</table>

This proposal is analogous to that for dopamine receptors, wherein we suggest that 1-2 nM 3H-neuroleptic labels post-synaptic sites, while >2 nM 3H-apomorphine for 3H-noradpine binding. (Support by the MRC of Canada, the Ontario Mental Health Foundation, and the W. Garfield Weston Foundation.)


Striatal dopamine (DA) receptors activate an adenylate cyclase and have also been characterized by the binding of various radio-labeled ligands. A potent DA agonist, 2-amino-6, 7-hydroxy-1, 1,2,3,4-tetrahydroxypalmital (ADTN), has recently become available in a tritiated form (3 Ci/mmol). Rat striatal binding of ADTN is saturable and occurs rapidly at 37°C. 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 ADTN is ADTN (IC50 = 13 nM) >apomorphine >dopamine (IC50 = 400 nM) >noradrenaline >epinephrine >5HT. Among DA antagonists (+)-butaclamol (IC50 = 4 nM) >fluphenazine >chlorpromazine >haloperidol >spioperidol (IC50 = 250 nM) >promazine. The drug specificity of this binding site is similar to that of sites labeled by other DA agonists. The DA antagonist 3H-spioperidol (3H-SPIRO) also labels sites in rat striatum with a drug specificity similar to that of sites labeled by 3H-halo-peridol. Although little cortical binding of 3H-haloperidol is detectable, 3H-SPIRO binding in the cortex is 20% of striatal levels. DA (IC50 = 2.2 µM) is 20 times more potent than 3H in displacing striatal 3H-SPIRO binding, however, 3H (IC50 = 4.4 µM) is 50 fold more potent than DA in displacing cortical 3H-SPIRO binding. That 3H-SPIRO labels 5HT receptors is supported by 3H-SPIRO binding in hippocampus which has a large 5HT but no DA innervation, and the high affinity of 3H-SPIRO for 5HT receptors previously published by others.
1684 REDUCTION IN STEREOSPECIFIC BINDING OF 3H-DIHYDROMORPHINE IN CNS REGIONS OF AGED RATS. Beatriz J. Vasquez, Vina R. Spehler*, Rite B. Meising, Robert R. Jensen, Joe L. Martinez, Jr., and James L. McLaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

There is extensive evidence that neurotransmitter 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 0.2M sucrose. 1.8 ml of 0.05M tris buffer, pH 7.4, to determine stereospecific binding of 3H-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.

<table>
<thead>
<tr>
<th>Region</th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral hemispheres</td>
<td>61±2.3</td>
<td>51±3.27*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>19±1.43</td>
<td>15±1.11*</td>
</tr>
<tr>
<td>Striatum</td>
<td>56±3.74</td>
<td>45±2.33**</td>
</tr>
<tr>
<td>Amygdala</td>
<td>37±4.18</td>
<td>36±3.0</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>30±2.27</td>
<td>30±2.75</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>14±1.68</td>
<td>12±2.09</td>
</tr>
<tr>
<td>Midbrain</td>
<td>24±2.90</td>
<td>22±1.50</td>
</tr>
<tr>
<td>Pons medulla</td>
<td>15±1.40</td>
<td>17±1.50</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>7±0.38</td>
<td>7±0.86</td>
</tr>
</tbody>
</table>

* 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. Support: NIH grants AG 00469, MH 05358 (R.A.J.); BNS 76-17370; and the McKnight Foundation.

1685 DECREASED ß-ADRENERGIC RESPONSES IN RAT CEREBRAL CORTEX FOLLOWING CHRONIC ESTROGEN TREATMENT. H. Ryan Wagner*, Keith A. Crabich 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 (substitivity) in postsynaptic ß-adrenergic receptor cyclic sensitivity, we began to study the effects of chronic estrogen exposure on this system. Six-week old female Sprague-Dawley rats were bilaterally ovariectomized and and sold dietary and aging diets. The rats were killed by decapitation (three weeks) all rats were implanted with a silastic pellet containing ethynylestradiol or nothing. Twelve to fourteen days after receiving the pellets, rats were killed and cerebral cortices from each group were homogenized and pooled. Approximately 1/2 of the tissue from each group was homogenized, made into membranes, and assayed for cyclic 3',5'-adenosine monophosphate (cAMP) in response to various concentrations of the ß-adrenergic agonist, (-)-isoproterenol (ISO). Maximum accumulations of cAMP in the presence of 10 nM ISO were reduced in estrogen-treated rats (31 pmol/mg protein) compared to untreated rats (35 pmol/mg protein). The maximum density of [3H] DNA membrane binding sites was also decreased in ovariectomized females exposed to estradiol (145 fmoles/mg protein) compared to untreated rats (160 fmoles/mg protein). These results indicate that there is a significant decrease in ß-adrenergic mediated cAMP responses by both high in vivo estrogen levels, which were shown by isoelectric focusing to consist largely of ß-adrenergic membrane receptors, and that receptor changes reflect a decrease in maximum receptor number with no change in the affinity of the ligand for the receptor (Kd = 3.7 nM; control; Kd = 3.5 nM, estrogen). Preliminary studies in other brain regions showed that in these regions the density of ß-adrenergic receptors was also significantly decreased in the striatum, hypothalamus, and olfactory bulb (p < 0.05) but not in the cerebellum, hippocampus, or cortex. These data indicate 1) that ß-adrenergically mediated cAMP responses are reduced by high in vivo estrogen levels, 2) that this pharmacologically is mediated in a complex manner in ß-adrenergic membrane receptors, and 3) that receptor changes reflect a decrease in maximum receptor number with no change in the affinity of the ligand for the receptor. Although these data are consistent with an estrogen-induced increase in noradrenergic neuronal activity, we presently cannot exclude direct effects of estrogen on ß-adrenergic receptors or indirect effects on those same receptors resulting from estrogen-induced alterations in a second unspacific variable (e.g., weight, decreased body temperature) as alternative explanations. (Supported by VA 1680, NIH NS13101, NS06233, MH06058, and AG00029.)


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 innervated normal rat muscle (Almon and Appel (1975) Biochim. Biophys. Acta 393, 60). 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 125I-m-hungaroroxin and receptor, addition of 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 against 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 rat electric organs 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. The results 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.


It has recently been found that 6-hydroxydopamine-induced lesions of the nigro-striatal neurons resulted in a 50% reduction in the number of striatal dopamine receptors and that the number of sites for 3H-apomorphine or 3H-spiperone in the contralateral unlesioned striatum when compared to unlesioned or intrastriatally saline injected animals (Fig.). This indicates that the concept of 3H-apomorphine labeling pre-synaptic receptors, while 3H-spiperone labeling post-synaptic dopamine receptors is incorrect. These results thus suggest a bilateral communication between the two striatata, as postulated by Piechowall et al. (Supported by MRC and OMHF).

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, University of Washington, Seattle, WA 98195.

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 illumination of the spindle, tension 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, and phase, A, of the receptor potential and tension (with respect to displacement) of the first harmonic responses were used to construct Bode plots. Measurements were performed under linear response conditions, as described below.

For f below approximately 10 Hz, A was constant or showed a slight monotonic increase with increasing f, while A was 10^(-5) 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 was an approxi mately power-law function of f, A = k f^n, with n = 0.3-0.5, depending on the particular spindle. A was 30-45° at low f, usually rose gradually with f to a peak at approximately 2 Hz, then decreased slowly with further increases in frequency to approximately 15 Hz. Neither A nor A showed a tendency to approach 0° as f approached 0.01 Hz, the lowest attainable measurement frequency.

In the range from 1.0 to approximately 10-25 ± peak-to-peak, A and A did not depend on the amplitude of stretch and harmonic plateau in the response waveforms was negligible. Frequency response measurements were obtained at constant amplitude within this range, determined independently for each spindle. Higher order amplitudes were amplitudes A, A, and A, increased with increasing amplitude as a power-law function, independent of frequency. Harmonic distortion increased with amplitude, and higher harmonics. With the tension nor receptor potential responses were consistent with simple linear models employing finite combinations of visco-elastic elements.


The extent to which 3-amino butyrate is bound to rat brain synapti c 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 the uniodinated membrane at 0.25 µM 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 µM 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 distribution which allows for the existence of several kinds of non-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.
EFFECTS OF CHRONIC ALTERATIONS IN RECEPTOR STIMULATION ON 8-ADRENERGIC RECEPTORS IN RAT BRAIN. B.B. ULBRICH, D.A. STAUNTON, P.H. GROVES AND P.B. MOLINOFF. DEPT. PHARMACOL., UNIV. COLORADO MED. CTR., DENVER, CO 80262.

Access of norepinephrine to postsynaptic 8-adrenergic receptors can be altered by the chronic administration of various pharmacological agents. The effect of such agents on adenylate cyclase was measured by the in vitro assay of adenylate cyclase in the presence and absence of various purine nucleotides. In striatal membranes GTP (0.3mM) produced a 4-5 fold decrease in the affinity of 8-adrenergic receptors for agonists. The affinities for antagonists were not affected. In the current studies inhibition of the binding of labelled spiroperidol for sites on striatal membranes [control: Kd=79±6 pM; GTP: Kd=85±5 pM]. Similar inhibition of the binding of spiroperidol by the dopamine receptor antagonists (+) and (-)-butaclamol or (a) and (b)-fluphenazine. 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 that include another receptor whose effects may be mediated through changes in adenylate cyclase activity. In contrast to the agonist-specific effects of GTP in the striatum, addition of GTP to membranes prepared from frontal cortex had no effect on the inhibition of adenylate cyclase by either DA, 8-adrenergic receptors, 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 site 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.

EFFECTS OF GTP ON BRAIN DOPAMINE RECEPTORS. M. A. ZAHNISER, D. A. STAUNTON, AND W. D. DiBiVER. 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 for agonists and 8-adrenergic receptors for agonists. The affinities for antagonists were not affected. In the current studies 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) decreased the affinity of spiroperidol for 8-adrenergic receptors. The affinity of spiroperidol for sites on striatal membranes [control: Kd=79±6 pM; GTP: Kd=85±5 pM]. Similar inhibition of the binding of spiroperidol by the dopamine receptor antagonists (+) and (-)-butaclamol or (a) and (b)-fluphenazine. 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 that include another receptor whose effects may be mediated through changes in adenylate cyclase activity. In contrast to the agonist-specific effects of GTP in the striatum, addition of GTP to membranes prepared from frontal cortex had no effect on the inhibition of adenylate cyclase by either DA, 8-adrenergic receptors, 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 site 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.


The binding of 8 nerve growth factor (NGF) to nuclei from dorsal root ganglia was observed by Andres, R.T. et al. (PNAS, USA 74: 2685, 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 NGF in culture by the outgrowth of neurites. When PC12 cells were grown in culture in the presence of 50ng/ml NGF 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.
REGENERATION
CELL-TO-CELL SPECIFICITY OF REGENERATED GIANT AXONS IN EARTHWORMS. Stewart C. Biret 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 (Biret 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 (Biret and Bittner; Fed. Proc. 36:1554, 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 these 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 HgCl2, developed with ammonium sulfide, embedded in paraffin, sectioned at 10 microns, and the cobalt stain enhanced with the Tisch’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 NCDA to G.D.B.)

OLFACTORY NERVE REGENERATION IN THE AXOLOTL FOLLOWING NERVE GROWTH FACTOR (NGF), ANTI-NGF, AND D-AMPHETAMINE TREATMENT. P.M. Adams, H.W. Hejlingson, J.R. Perez-Polo and K. Hall
Dept. of Psychiatry and Behavioral Science, and Pharmacology, Anatomy and Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550

In previous work we described the extensive central nervous system regenerative capacity of the axolotl (Ambystoma mexicanum). We have studied this regeneration following a full transection 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, on nerve regrowth. The NGF or anti-NGF were administered intracranially immediately after surgery. The d-amphetamine was administered chronically beginning immediately or Day 5 post-trauma 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 over larger fibers in the NGF treated animals. In contrast, 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 was supported by Grant Nos. DEEM 5501-RR-0947-16 (G.D.B.) and Research Career Development Award 1-K01-NS-00213.

RETENTION OF SPECIFICITY FOR APPROPRIATE SYNAPTIC SITES BY REGENERATING FROG OPTIC AXONS. Ronald C. Hofer and Dennis J. Stelmaer
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 regenerating their normal targets. Recent investigations have demonstrated that by ablation of normal visual targets (sectum) 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 (Kail 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 4 months, the right eye of each frog was injected with 4 µl of 125I-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 target areas 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 control regions even though normal optic nerve projections remained intact. Examination of the distribution of label within contralateral thalamic visual target areas 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 control regions even though normal optic nerve projections remained intact.

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, '76). 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 midpoints between the initial crush and the eyeball and 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. Examination of the retinal ganglion cell layer of animals treated to delay axon outgrowth demonstrate prominent nucleoli and chromatins 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.)
THE EPENDYMAL CELLS OF THE SPINAL CORD IN RAT AND STINGRAY Richard E. Craggoshall and Robert R. Leonard, Deps. 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 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, the opinion has been modified because it has been shown that a population of small neurons in the forebrain keeps proliferating into adult life. Nevertheless, it is still felt that the number of synapses 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 that the fact that these axons 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 epidermal cells of the spinal cord were examined. The epidermal cells of the stingray can be divided into 2 populations, first a typical columnar cell that forms the bulk of the epidermal 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 epidermal 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 epidermal cell types as described above. It was of interest, however, that it seemed to be synapses on the typical epidermal cells in this animal. Synapses have previously been described in the tanyocytes of the 3rd ventricle in mammals, but these are not described, to our knowledge, in the spinal cord. The relation of these findings in stingray and rat to the question of proliferation of non-epidermal neuronal populations will be discussed. Supported by grants NS 11255, NS 10161, The Muscular Dystrophy Society of America, and NIH Fellowship NS 0164.


Following denervation by crushing the sciatic nerve, fast motor axons regenerate faster than slow typical axons and re-innervate non selectively twitch and slow muscle fibers. Slow axons reach the muscle later and re-innervate selectively slow muscle fibers (Crispim & 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 impaled with two intracellular microelectrodes and were characterized as twitch or slow fibers 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 response by threshold stimulation to the proximal and to the distal 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. 2 slow fibers studied 9-46 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. > 18 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.
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 neurite outgrowth, provided that the optic nerve with hemisphere regeneration complete by six weeks post-surgery. The axolotl (Ambystoma mexicanum) is a neotenic amphibian with an extensive regenerative capability within the central nervous system. 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 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 examined), unique markers of the boundaries of the original lesion.

HISTOLOGICAL EVIDENCE FOR AXONAL REGENERATION IN BRAINS OF ADULT RATS. Anne P. Foerster. Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada, M5G 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 about 200µ beyond the horizontal cutting wire. The device was lowered through the brain (adult male rats, 350-400g) by a micromanipulator, and the two protruding vertical wires were cemented in 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 "wounds" 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 then stained horizontally or vertically with Luxol Fast Blue and Nuclear Fast Red. Because of their predominantly parallel-fibred alignment, the horizontal parasagittal sections of the 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 3-4 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 closely apposed on 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 were evidently undergoing a normal axon resurgence and relative mobility 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 using a cutting cross-wire. When a tract lesioned 18 or more days earlier had failed to have detoured in this characteristic manner, measurements indicate that the cut had extended more than about 200µ outside its borders.

The axolotl (Ambystoma mexicanum) is a neotenic amphibian with an extensive regenerative capability within the central nervous system. 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 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 examined), unique markers of the boundaries of the original lesion.


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 at Golchi stages. Post-crushing studies indicate that the retinal tubulin content does not show a comparable increase, suggesting that the altered labeling represents regeneration of new tubulin, or tubulin was increased transport of newly synthesized tubulin out of the retina.

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 about 200µ outside its borders.

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 1% of the labeled soluble protein in contralateral tecta from control fish, whereas, in the post-crush fish, 36% of the labeled soluble protein corresponded to 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.


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 at Golchi stages. Post-crushing studies indicate that the retinal tubulin content does not show a comparable increase, suggesting that the altered labeling represents regeneration of new tubulin, or tubulin was increased transport of newly synthesized tubulin out of the retina.

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 about 200µ outside its borders.

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 1% of the labeled soluble protein in contralateral tecta from control fish, whereas, in the post-crush fish, 36% of the labeled soluble protein corresponded to 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.
A COMPARATIVE STUDY OF CNS NEURON REGENERATIVE ABILITIES: Claire E. Holmbach* 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 vertebrates 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 capacity to regenerate. 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 Macrodabella 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, our results which do add CNS neurons during ontogenesis 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 NEDA NS-00070 to G.D.B.)

FURTHER EVIDENCE THAT 4 S 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 3H-uridine into the eye of goldfish, 3H-RNA is found within regenerating optic axons in the optical tectum (Gambetti, et al., 1976, Brain Res. 112: 375-381). This implies that 3H-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 days later 3H-uridine was injected into both eyes of the fish, and the 3H-RNA was isolated from both optic nerves. It is likely that this RNA was synthesized in retinal ganglion cell bodies in the eye, and then transported axonally into regenerating optic axons. If 4S RNA is the only RNA transported in this system, we would predict that the 88% decrease in transported RNA would be a greater loss in transection RNA than in ribosomal RNA. Polyacrylamide gel electrophoresis (PAGE) of tectal RNA following the procedures outlined above, showed that ribosomal 3H-RNA was decreased by ~70%, whereas 4S RNA was decreased by ~90%. These results are interpreted as evidence that the decrease in tectal RNA is due to a decrease in the availability of 3H nucleotides for tectal RNA synthesis, and that the decrease in 4S RNA is due to the same process plus a loss of axonally transported 4S RNA. In the second experiment, with optic nerves transected, were crushed in 36 fish and 18 days later 3H-uridine was injected into both eyes of the fish. One group of 12 fish was killed 6 days later and tectal 3H-4S RNA was characterized by PAGE. The second group of 12 fish had both optic nerves cut 6 ds. after injection and killed 7 ds. later, allowing for optic nerves to the tectum to degenerate. The 3H-RNA with degenerated optic axons. Group III was killed along with Group II and tectal 3H-4S RNA was analyzed by PAGE. If 4S RNA is intra-axonal we would predict that 4S RNA in Group II compared to Groups I and III. In Groups I and III, approx. 70% of tectal 4S RNA was 45S, whereas in Group II (tecta in which optic nerve RNA was isolated). It is likely that the loss of 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.

UNIVERSITY OF CHICAGO PRESS
1711 PROTEIN SYNTHESIS AND FAST AXONAL TRANSPORT IN REGENERATING GOLDFISH RETINAL GANGLION CELLS: EFFECT OF A CONDITIONING LESION. 
Irving G. McQuarrie and Bernice Graffstein. 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 smaller 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-transported protein; axonal outgrowth was slower than in the conditioning lesion group.


The influence of the supraspinal ganglion on the withdrawal responses of the earthworm (L. terrestris) was studied 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. The withdrawal primarily resulted from a decrease in sensitivity of the photoreceptor cells. Although several methods of conditioning were used, the results indicated that the axons reaching the target 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-transported protein; axonal outgrowth was slower than in the conditioning lesion group.


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 ofependymal regeneration 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 in the light and electron microscope during post-operative intervals between 2 to 6 weeks. Extensive maturation of the reconstituted cord had occurred 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, epineuronal 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 mm 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 premetamorphic and early postmetamorphic stages of development by the time they were sacrificed. As seen in a companion study of normal spinal cord maturation and with respect to the rostral 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-11836 and the Paralyzed Veterans of America)


To study the factors which govern how regenerating nerve terminals recognize and re-establish synapses with target cells, we have attempted to reinnervate parsympathetic 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 the regenerating synapses. One of the target cells in this preparation is the frog preganglionic axon which terminates in boutons on the cell bodies of cardiac ganglion cells. These synapses are cholinergic and occur fairly readily in dissociated cultures. We hope to exploit the properties of these 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 stumps of the left vagus and hypoglossal nerves were 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 indicated by a 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 all of the preganglionic cells (the action potentials received suprathreshold responses). The conduction velocity of axons reinnervating the ganglion from the hypoglossal nerve was about 0.5 m/sec. Light (1kHz, 1-msec) and electron microscopic evidence showed that the hybrid synapses looked more like the preganglionic than like vagal boutons and unlike motor end-plates. In addition, axon studies showed that the hybrid synapses resembled the ones which had been shown to be innervated by preganglionic axons and exhibited the properties of autonomic ganglia. We then tested the hypothesis that the preganglionic axons retained their transmitter receptor characteristics.

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 high degree of precision. As a next step in studying the mechanism of sprouting 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 noose may be removed by 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 slips coated with polylysine they adhere to the surface and send sprouts and to send 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, noninverting electrical connections. 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.


Most workers examining reinnervation of mammalian skeletal muscles conclude that the process is non-selective. However, Hoh (J. Physiol. 251, 1975) reported that the rat slow nerve fibers 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 reinervate 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 reinervate the EDL muscle under optimal conditions, i.e., no competition from the EDL nerve and the soleus muscles were removed completely to prevent self-reinervation. Three to 8 wk 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 EDL muscle. Functional innervation of individual EDL fibers was assessed by the oxygen exhaustion technique of Kugelberg and Edström (J. Neurol. Neurosurg. Psychiat. 31, 1968). Serial cryostat sections were stained histochemically with the PAS reaction for glycogen and by myosin ATPase and NADH dehydrogenase activities to identify fiber types and assess the functional state of their innervation. At 3 wk only EDL muscles contracted in response to soleus nerve stimulation. However, 5-8 wk 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 synapticizing. Thus, these results indicate that the incomplete reinnervation of the EDL in part accounted for by the failure of soleus fibers to reinervate 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. Amer., Inc.

PEENETRATION OF IMPLANTED ASTROCYTIC SCARS BY REGENERATING AMPHIBIAN OPTIC NERVE FIBERS. Paul J. Reier, Department of Anatomy, University of Maryland School of Medicine, Baltimore, Md.

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. We report here that in the regenerating optic nerve of the newt, Necturus maculosus, 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 reactive astrocytic scar. Glial scars were formed in the optic nerves of post-metamorphic, juvenile Xenopus by unilateral encuclutures. 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 transplanted 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. This study is consistent with the notion that self-reinnervation of the EDL 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 reinervate 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 reinervate the EDL muscle under optimal conditions, i.e., no competition from the EDL nerve and the soleus muscles were removed completely to prevent self-reinervation. Three to 8 wk 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 EDL muscle. Functional innervation of individual EDL fibers was assessed by the oxygen exhaustion technique of Kugelberg and Edström (J. Neurol. Neurosurg. Psychiat. 31, 1968). Serial cryostat sections were stained histochemically with the PAS reaction for glycogen and by myosin ATPase and NADH dehydrogenase activities to identify fiber types and assess the functional state of their innervation. At 3 wk only EDL muscles contracted in response to soleus nerve stimulation. However, 5-8 wk 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 synapticizing. Thus, these results indicate that the incomplete reinnervation of the EDL in part accounted for by the failure of soleus fibers to reinervate 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. Amer., Inc.

NEONATAL 6-HYDROXYDOPAMINE-INDUCED NORADRENERGIC SPROUTING IN THE RAT CEREBELLM. INTRACISTERNAL DOSE-RESPONSE STUDIES. Richard H. Schneider and Ramnik 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 degeneration of the noradrenergic locus coeruleus system. This is characterized by extensive, permanent loss of norepinephrine (NE) from telencephalic and diencephalic structures and a dramatic decrease in NE content in the brain stem and cerebellum. As a part of a more extensive study of the reasons for this developmental response to 6OHDA, a dose-response relationship for the effect of intracisternal 6OHDA application in order to obviate any influence of the blood brain barrier. On the day of birth the dam-bred Sprague-Dawley rat pups were distributed 10 per litter and injected intracisternally with 10, 20, 40 or 50 µg 6OHDA or 4 µl vehicle while cold-anesthetized. At 32-35 days of age all animals were subjected to a radiolabeled method. The cerebellum was cut in a cryostat in 50 µm thick sagittal 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 region NE depletion was 50-75% after 10 µg, and greater than 90% at the 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 120-255%, 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 venous lobules I-VI and lowest in venous lobules VII-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 differentiation is not apparent. In Xenopus tadpoles, such scars consist of an extremely dense glial scar formed by mature, 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 astroglial scarring in the failure of mammalian CNS regeneration. (Supported by NIH Grant NS-13836 and the Paralyzed Veterans of America).
Preliminary Studies of Protein Synthesis in the Frog Retina 1-6 Days After Section of the Optic Nerve. Thomas M. Scott and Alan 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 inability of the axotomized neuron to synthesize protein necessary for re-growth. 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 rats. At daily intervals from 1-6 days, each animal was injected I.V. with 30μCi [3H]-leucine. Three hours later the animals were decapitated and the retinas removed into 3ml of 0.9% saline. The retinas were homogenized in a glass homogenizer after removal of protein precipitates. The homogenate was then added to the homogenate. 20 minutes later the homogenate was filtered through a millipore filter. Ten millipore filters were then added to the TCA precipitable fraction between 4 and 6-fold, 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 50% of homogenate removed before TCA precipitation was 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. Incorporation figures for the TCA precipitable fraction were determined by comparing the amount of protein 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 a tendency for an increase in transport, perhaps reflecting an increase in incorporation rate into the TCA precipitable fraction between 4 and 6-fold, 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 50% of homogenate removed before TCA precipitation was 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. Incorporation figures for the TCA precipitable fraction were determined by comparing the amount of protein 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 a tendency for an increase in transport, perhaps reflecting an increase in incorporation, 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.


Nerve growth factor (NGF) treatment, given as a single 200 BU intracocular 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) peaks 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 clearance of 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 pre-reactive state for 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 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 nuclei 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 ganglion cells per 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 Grant Research 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-NHS Grant NS 12070 awarded to D. Turner. D. Turner is also the recipient of an NIH Research Career Development Award NS 00338.


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 deenveinated ganglia from 150-175 g rats (4-6 weeks of age) were placed in stainless steel Petri dishes and placed in 37°C bath with the following media: Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum. Incorporation of leucine-14C 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. NADH/NAD 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 Kaufman, Brain Res. 55:141-157, 1974) are maintained in culture as indicated in the table below.

<table>
<thead>
<tr>
<th>culture</th>
<th>6PGDH activity</th>
<th>protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.3 ± 0.15</td>
<td>62.5 ± 2.0</td>
</tr>
<tr>
<td>48 NGF</td>
<td>8.81 ± 0.26</td>
<td>85.7 ± 2.0</td>
</tr>
<tr>
<td>48 -NGF</td>
<td>10.68 ± 0.53</td>
<td>71.8 ± 3.0</td>
</tr>
</tbody>
</table>

The increase in 6PGDH activity that occurs in normal ganglia placed in culture is significantly greater than the increase in culture activity, 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.


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 Marcalne 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. All parallel series was assayed for the activity of choline acetyltransferase (CAT), a marker for formation of cholinergic synapses. CAT was 25% control by day 4 and 65% 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. Neuro. Sci., 34:373, 1977). For maximal functional development of transplanted muscle fibers, efforts should be directed to facilitation of reinnervation. This support is provided by grants from the Muscular Dystrophy Assn., Inc., NIH - NS 13116 and the National Amyotrophic Lateral Sclerosis (ALS) Foundation.
POLYACRYLAMIDE GEL SEPARATION OF AMINO ACID LABELLED PROTEIN FROM BRAIN AND SPINAL CORD AFTER SPINAL CORD HEMISECTION IN THE RAT. M. N. Wells, Dept. of Neurosci., Univ. of Fla., Gainesville, Fla.

Following spinal cord hemisection in the rat, the general increase in amino acid incorporation into protein occurs 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 duro cut (sham) at spinal segment T2, a left spinal cord hemisection, or no operative stress (Wells and Bernstein, Exp. Neurol. 57: 900-912, 1978). Samples were taken from somatosomal cortex and left side of spinal cord extending 2 mm rostral and caudal to the lesion. Samples were prepared for electrophoresis and protein separated on 10.0% gradient (7.5-18.75% acrylamide), 38% polyacrylamide slab gels. Gels were fixed in trichloroacetic acid, stained with Coomassie blue, photographed, cut into slices, and processed for scintillation counting.

In somatosomal 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. The nerve allograft at 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 56 days postoperative, significantly higher 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.)

FUNCTIONAL REPAIR OF INJURED PERIPHERAL NERVE TISSUE WITH NERVE ALLOGRAFTS BEARING MAJOR AND MINOR TRANSPLANTATION ANTIGENS. Andrew A. Zalewski and Willy S. Silvers*. UMC, NICHD, NIH, Bethesda, Md. 20014 and Dept. of Human Genetics, Univ. of Pa. Sch. Med., Philadelphia, Pa. 19104

We previously reported that neurilemmal (Schwann) cell survival was prolonged in allografts which contained minor rather than both major and minor transplantation antigens. Since the neurilemmal cells of a nerve allograft are permissive and may be replaced by host nerve cells, we investigated whether host motor nerve fibers would regenerate through a nerve allograft and innervate 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 neuromuscular junction causes a contraction of the EBD and TA muscles which return to normal in three to four weeks in sham operated animals. In the functional assay of injured sciatic nerves, the EBD and TA muscles were significantly smaller in the operated animals. In the functionally denervated sciatic nerves, the EBD and TA muscles were similar in size to the normal uninjured muscles. The present study was designed to determine whether regeneration of the peripheral nervous system could occur in a nerve allograft when the host nerve donor was killed. The animal model utilized was the tibial nerve of allografted animals caused a contraction of the EBD and TA muscles which may be caused by a combination of factors: the presence of nerve fibers, neuromuscular junctions, and large-diameter muscle fibers which were localized into larger type II fibers. The present study was designed to determine whether regeneration of the peripheral nervous system could occur in a nerve allograft when the host nerve donor was killed. The animal model utilized was the tibial nerve of allografted animals caused a contraction of the EBD and TA muscles which may be caused by a combination of factors: the presence of nerve fibers, neuromuscular junctions, and large-diameter muscle fibers which were localized into larger type II fibers.
SLEEP
1726

THE EVOKED POTENTIAL SOMNOGRAM - HUMAN AND ANIMAL STUDIES. R.G. Bickford, R. Hajdukovic*, K. Hanson*, Y. Kamber*, C.B. McCutchen, and J. Frankel. Department of Neurosciences, EEG Laboratory, University of California, San Diego, La Jolla, CA and the Veterans Administration Hospital, La Jolla, CA 92039.

The somnogram (K. Hanson, et al., Proc. San Diego Biomed. Symp., 13:545-546, 1974) is a computer generated (PDP/12 and PDP/140) 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 68 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 clicks, 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) monotonous 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

1727

CIRCADIAN RHYTHMS OF SLEEP, ACTIVITY, AND TEMPERATURE IN THE RAT UNDER ENTRAINED AND FREE-RUNNING CONDITIONS. Charmaine Eastman. Sleep Lab., Univ. of Chicago, Chicago, IL 60637.

Long-term recording 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 oscillators(s). Sleep and waking were scored in 30 sec epochs by the computer system of Bergmann et al. (Sleep 3:247, 1980). Temperature was recorded from thermistors implanted in the intra-peritoneal 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 beat-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.

Figure 1. Onset of sleep in normal subject; 30 minute recording.

1728


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 in seeing whether DL-$\beta$-(naphthyl-1)-alanine hydrochloride ($\beta$-NA), a tryptophan analog, had similar effects. In one group of adult male rats we analyzed 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and norepinephrine (NE) in the cortex (CX), hippocampus (HIP), pons-medulla (PM) and striatum-thalamus (ST) first forty-five minutes and forty minutes after a single injection of $\beta$-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 HIP 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 $\beta$-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. Pooled analysis performed at half-hour intervals showed that $\beta$-NA increased SWS by 11% and decreased W by 11% during the first half-hour period. We also found that $\beta$-NA reduced SWS latency by 50%. There was no change in PS or PS latency. Our data show that SWS latency is controlled with the rate of DA, NE, and 5-HT. Since increased 5-HT turnover has been found to correlate with SWS (Buckingham and Radulovacki, 1975) Kovačević 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 HIP.

1729

METHOD FOR INTRACELLULAR RECORDING OF LUMBAROSAL MOTORNEURONS DURING NATURAL SLEEP IN CATS. Loyd L. Glenn, Arthur Youts and William G. 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 lumbar motoneurons of unanesthetized and unparalyzed cats. Cats were chronically instrumented for sleep recordings and stimulation of the tibial nerve. The lumbar and pedicles of vertebrae L4-L6 were exposed and stainless steel screws inserted into the body of each vertebrae. 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. Anatomically, a cylinder can be sealed with an electrode opening. After recovery from surgery, the cats were periodically habituated to simultaneous cranial and lumbar restraint prior to any microelectrode deserts. Although it was possible to isolate anterolateral and ventral columns, 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 lumbaroscal 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). Some 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. Motor units of these motoneurons 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.

Administration of benzodiazepines produces a decrease in brain dopamine (DA) metabolism while data on brain 5-hydroxytryptamine (5-HT) metabolism are inconclusive. We examined the effects of various doses of diazepam (DZ) on sleep, cerebral blood flow (CBF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA), a 5-HT metabolite, and homovanillic acid (HVA), a DA metabolite, and rectal temperatures of cats. CBF was obtained from a cannula in the cisterna magna (Radulovacki, 1974). 5-HIAA and HVA were determined by the method of Korf and Valkenburg-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 of 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. Diazepam 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.


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. Post-central neuronal response to the amplitude of a sine wave mechanical stimulus and neuronal spontaneous activity were observed repeatedly during all the phases of normal night sleep cycles. 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 (S5,6), to 64 ± 26% during deep sleep (S5,6), and to 94 ± 98% during desynchronized sleep with rapid eye movements (REMs).

The responsiveness of neurons of the primary sensory cortex (Brodman area 1,2,3) appears to be a balance of the specific thalamocortical input versus the general 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.


5-Hydroxytryptamine (5HT) has been implicated in the mediation of sleeping behavior (Jouvet, 1969). The exact role of 5HT in sleep mediator probably involves on increased turnover of the amine at cortical 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 antidepress the effects of 5HT action.

Two groups of cats were surgically prepared with EEG, EMG, and EOG leads to evaluate sleep stages 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).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total % W</th>
<th>Total % SWS</th>
<th>Total % PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo + ME</td>
<td>67.8 ± 6.8</td>
<td>32.7 ± 3.4*</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>L-TRYP + ME</td>
<td>65.2 ± 7.8</td>
<td>34.8 ± 3.2*</td>
<td>1.5 ± 0.6</td>
</tr>
</tbody>
</table>

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
LESSIONS OF NON-MONOMINE NEURONS OF THE PONTINE TEGMENTUM ELIMINATE PARADOXICAL SLEEP IN THE CAT. Barbara E. Jones. Deps. Neurol., Neurosurg., and Psychol., McGill Univ., Montreal Neurological Institute, Mtl., P.Q., Canada R3A 2N4. 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 Ergebneisse Psychol. 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 monoamine neurons damage only the locomotor center, they do 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.

It was found that spontaneous PGO spikes were evoked in the 2 animals which did not exhibit spontaneous PGO spikes. The mean amounts of sleep stages zero (wake), and 1, 2, and 3 combined were significantly less than those of normal control subjects. The results suggest that the pontine FTG, and noradrenaline 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.

SLEEP PATTERNS IN QUADRILEGIC PATIENTS
Lawrence W. Kreisley MD. From the Research and Spinal Cord Injury Services, West Haven Veterans Administration Hospital, West Haven, Conn., and the Department of Neurology, Yale Medical School, New Haven, Conn., and the Department of Neurology, Children's 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 12 quadriplegic patients. The subjects, who were evaluated for this report, had no abnormal EEG findings, and 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. They were studied for two consecutive 24-hour 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, 2, and 3 were substantially less than those of normal control subjects (p<.05). 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 (p<.05). One explanation for the increased amounts of waking (stage zero) and light sleep (stage 1), and the diminished REM sleep, 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 in locomotor inactivity and immobility due to motor paralysis may be an important factor in reducing the "deeper" phases of sleep (stages 3 & 4), since increases may increase the amount of waking stages in the deep of normals. These studies indicate that the sleep patterns of individuals with cervical spinal cord trauma are dramatically and persistently altered.


MULTIPLE SLEEP LATENCY TEST IN NORMAL AND NARCOLEPTIC DOGS

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 normal, 2 narcoleptic) were surgically prepared for chronic sleep recordings. Following a 2-week recovery period, one affected and one normal dog were placed in separate recording chambers 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 followed by habituation of 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. In comparison to control data, latencies to sleep tended to be shorter in affected dogs than normals 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. 1.7 ± 2.6 4.3 ± 3.5
Control 2.4 ± 1.0 8.1 ± 8.3
Poodle Narc. 1.7 ± 2.6 1.1 ± 1.9
Control 2.8 ± 3.3 21.5 ± 12.4
Beagle Narc. 3.3 ± 2.4 3.8 ± 3.6
Control 10.7 ± 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 nonspatial thalamic nuclei were recorded chronically in freely moving cats, using "floating" microelectrodes. The long trains of up to 100 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. Neurol. 1:259,1972). 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 theoretical 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 centromedian-parafascicular complex (CM-PF), only one fired during SWS in a manner consistent with theoretical distribution of patterns and independent of the 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 region of the 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.


Electrolytic lesioning of the central and descending vestibular nuclei has been reported to eliminate bursts of phasic activity (PSG spikes and associated automatic and spinal reflex modulations) during paradoxe sleep (PS) in dogs. Lesions made in the difficulty in creating complete, replicable lesions of the vestibular nuclei, this method of lesioning usually destroys a great deal of nonvestibular tissue. The purpose of the present study was 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, EOG, KCO and LIG activity were administered streptomycin subcutaneously over periods of time ranging from 4--24 days. The total amount 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 process. The 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, diarrhea 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, the cat, streptomycin effects differential actions in behavior and electrographic activity. The behavioral signs included significant ataxia and vestibular mechanisms. Electrophysiologically, increased amounts of slow activity are present in the EEG during sleep, but the same, on PS is less evident. The LGN was recorded at the level of the LGN is enhanced during slow wave and paradoxical sleep, and bursts of such activity persist.

Previous work in this laboratory with L-DOPA and a-dopethamine (Radulovitch et al., FASEB, 1978) has shown that these two agents increase wakefulness (W) and can partially substitute for paradoxical sleep (PS) rebounded in PS-deprived (PSD) cats. We have now studied the effects of CB between decreased PS and an increase in brain dopamine (DA) following the administration of a DA β-hydroxylase inhibitor diethyl dichlorocarbamate (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, diethyl dithiocarbamate (DDC) in RPO, RPC and MNGC 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 where their 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 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 CB and FL, respectively. Groups three and four, pretreated with FL, received either CB, respectively. After drug conditions were administered, EEG and EMG wave forms were continuously monitored for thirty hours. All records were obtained 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.

It was 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 rebounded, all 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.

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. Siegel II and S. Wilson, Dept. of Physiology and Biophysics, Univ. of Louisville Health Sciences Center, Louisville, Kentucky 40223.

TRIENED CATS. J. M. Siegel, D. J. McGinty and S. M. Breedlove* , Sepulveda V. A. Hospital, Sepulveda, CA 91343.

1744 SLEEP AND WAKING ACTIVITY OF NUCLEUS RETICULARIS PONTS 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 nucelicus reticularis pontis oralis-caudalis (RPO-RPC) and medullary nucleus gigantocellularis (MNGC) occupy the medullary reticular formation areas anterior and posterior to the pontine nucleus gigantocellularis (PNGC). Carli and Zanchetti1 identified the PNGC by the RPO-RPC region as the area where REM sleep occurred. Netick, Orem and Dement2, 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 characteristics of these cells 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 area1. Type 1 cells had no spontaneous activity during quiet waking and REM sleep. Type 2 cells had spontaneous activity during quiet waking 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 the medullary region that discharge selectively in REM sleep. Our results are consistent with a hypothesis of medial reticular formation involvement in the motor activation commonly seen in 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.


1745 VISUAL PATHWAYS MEDICATING THE EFFECTS OF LIGHT ON PARADOXICAL SLEEP IN RATS. C. S. Sisk and F. K. Stepaneh, 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 (Beyth, 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 (IOATP) 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 IOATP 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. Stephon and Zucker, 1972; Physiol. Beh. 8, 315-320).

These results suggest that neither the RHT nor the IOATP 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 reticular nucleus, optic tectum and the superior accessory optic tract in the effects of short dark periods on REM sleep.

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 al 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 μg/50 μl/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 μg. The effect started and finished immediately after the Carbachol push-pull injection. Doses larger than 30 μg produced an immediate blockage of the active units (satisfaction 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.
SOMATIC SENSORY SYSTEMS

Studies based on succinic dehydrogenase histochromy 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 corticothalamic 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 H-leucine or proline, bundles of labeled axons can be seen in the ventrobasal complex as early as the first postnatal day (birth±0). 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–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 segmental organization both cortical and trigeminal afferents to the thalamus. Supported by NSF grant GB41294 and NIMH grant MH81499-02.


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 the 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, neuronal damage to the ventrobasal cortex, the thalamus, or the somatosensory cortex (Killackey and Belford, Neurosci. Abst., 1976). In the present experiments, the effects of developing vibrissae representations to those in the trigeminal complex.

In the first part of the present study, littermate rats with normal vibrissae maintained on p. were sacrificed on Day 0 through 5. In the second part, littermate rats had a row or rows of vibrissae damaged at birth. These rats were also sacrificed 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 histochromy.

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 thalamus. The principal trigeminal 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 development of SDH activity is related to the damaged vibrissae. This shows a density of SDH activity much lower than normal. Further, the row of clusters normally seen in this area is reduced to a band of activity, but more often the area is amorphous. Finally, removal of vibrissae at different postnatal ages results in variations in the pattern as seen on 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 on Day 5. Removal of Days 0 through 2 leads to the pattern described above 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 or bands 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 MH 45999-02 and NSF GB 41294.)


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 cornea, the homolateral dorsal root, and the opposite homolateral dorsal root of 0–3°C. Rates of temperature change varying from 0.1°C to 3°C/sec were administered for a 3 sec duration producing stimulus intensities that ranged from 0.3°C to 6°C. Five stimulating surfaces (1.2, 4, 7.5, and 18 cm²) were introduced for between-magnitude estimates and comparison analyses. 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 subject 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 11210)

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 present study is the first of a series designed to determine whether or not these receptors are similar physiologically by examining their response characteristics. In this preliminary experiment the cornea of a lightly anesthetized rat (280–300 g) was removed and mounted in a lumen chamber by compressing the bulbar conjunctiva between a stainless steel ring and a glass 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 intracocularly. As many as five nerve bundles (100–150 um) 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 um dia.) Conduction velocity has not been measured. All units found have been recorded adapting a range from 0.5 mm from periphery to 0.5 mm from nasal to peripheral; the peripheral units are areas 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 temporal units are small (500 um dia) and located more toward the center of the cornea; they have little or no responsiveness to the calibrated hair but do respond to contact. Concluded: the presence there is functional evidence for more than one unit type in the rat cornea.

(Supported by NIH Grants EY 04108 and EY 00051)
1753 ACTIVITY PRODUCED THROUGH PERIPHERAL VASCULARIZATION 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 not 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 the thoracic 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 phenylphrine hydrochloride (PDG). Activity gradually returned 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 vasoconstrictor substances proposed by Kray and Acheam (Physiol. Rev. 26:183,1946). The location of the nerve corresponded to the distribution of the nerves to the superficial femoral artery described by Potts in human material (Anat. Nuz. 47:136,1916).

Thirteen times after occlusion of the vessel, it was identified which fired slowly and adapted prior to release of the occlusion. The nerve trunk had been divided centrally prior to the wall obstruction and thus the neuronal response might correspond to afferent reflex activity suggested by Haddy and Gibert (Circ. Res. 4:255,1976). One single unit was activated by function stimulation of an apparently rapidly adapting mechanoreceptor located on the saphenous artery 1.2 cm from the origin of the vessel. The unit responded 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 observed 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. Brounswig* (SPOR: E.F. Domnos). 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 squirrel monkeys (Sciurus niger) and Thirteen-lined ground squirrels (Spermophilus tridactylus) were examined in material prepared with Sevier and Hunger's (J. Neuropathol. exp. Neurrol. 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 (3.2 ± 0.5) than in ground squirrels (1.3 ± 0.3). Unexpectedly, the density of receptors in the palm region is significantly higher than in the digits for both squirrel species (48/mm² vs. 26/mm²). 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 activated 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 CYMOLOGOUS MONKEY. E. Burton and C.J. Robinson. Dept. 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 neuronal stimulation activates several regions around SII that do not receive projections from the ventroposterior nuclear region of the thalamus. In this study, the locations of cortically 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 tract. The granular insular cortex (Ig), which receives projections from the supragranular plate region of the thalamus, contains areas that primarily respond to stimulation of hairs over large (>100 cm²), generally bilateral receptive fields. These borders were often well defined. Neurons within the retrolateral insular cortex (RI), which receives projections from the posterior nucleus (PO), primarily respond to light tactile stimulation of rapidly adapting skin receptors, less than 10X responded to moderate or high threshold cutaneous stimulation. Receptive fields were of moderate size (<100 cm²), 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 is 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, were 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, the projection of cortical areas could only be made after correlating the recording sites with connective analyses in the same animal because of the great similarity in the somatic receptive properties in the make and unparalyzed animal. Consequently, previous physiological studies may have designated some of the somatic areas seen here as SII proper. (Sponsored by NIMH MH-08899 and OH-8127).

1756 ORIGIN OF PROPRIOCEPTIVE FIBERS INNERVATING THE TEMPOROMANDIBULAR JOINT CAPSULE. Norman F. Capra*, John R. Rohm* and Glenn B. Catterson. Dept. of Anit. and Pharma., Univ. MS Med. Center, Jackson, MS 39216. That the mesencephalic nucleus of the trigeminal nerve (MVN) mediates proprioreceptive impulses for the head region is generally accepted. In cats, we are unable by electrophysiological methods to identify MVN neurons that respond to passive movements of the temporo-mandibular joint. Unilateral injections of the retrograde axonal transport 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 contra-lateral 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 rostro-caudal extent of MVN revealed no labelled neurons in animals receiving unilateral injections of HRP in the temporo-mandibular joint. Labelled neurons were found only in the MVN 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 33-40 micrometers in diameter. These cells were located in the most caudal part of the mandibular division of the ganglion.

Somatotopically defined areas of the trigeminal ganglion is marked by 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.
In previous studies of adult rhesus monkeys, surgical lesions of the hand area of primary somatosensory cortex (SI) produced sensations and peramnestic deficits in the judgment of movement and tactile discriminations. Partial lesions of single hand representations in cytoarchitectural subdivisions of SI produced selective discrimination deficits and recovery of tactile function, 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 make tactile discrimination judgments.

The consequences of SI lesions in infants differed from those found in adult monkeys. Infants receiving partial lesions (Brodman's area 1) with ipsilateral somatosensory receptive fields (RFs) 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 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 of recovery of function suggests that the remaining SI cortex in the partial lesioned animals disrupts rather than facilitates the recovery process.

The results of these studies show that recovery of normal function following lesions of sensory cortex in infant primates. Although SI is functionally mature in the first weeks of life, it may have a greater capacity for regeneration than the discrimination function lacking in the adult and juvenile cortex. (Supported by PHS grants NS 12090 and NS 14261.)

The toposographic organization of cells in the lateral cervical nucleus (LCN) with ipsilateral somatosensory receptive fields (RFs) was examined with extracellular unitary recordings. Closely-spaced (100 μm grid) microelectrode penetrations were made, angling along the transverse axis of location of the LCN (dorsolateral to ventromedial) in the dorsolateral funiculus (DLF). RF location, stimulus sub-modality, antidromic latency (from the thalamus), and stimulus location were recorded for 34 LCN cells in six recording loci in four cats. LCN neurons with hindlimb (H) RFs were located dorsolaterally (superficially), those with forelimb (F) RFs 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 investigators of HRFs have concluded that these units were idiosyncratically related to other investigations. As it was thought possible to distinguish whether the underlying organization is "segmentomotopic" or "somatotopic". A special subpopulation of units (17%), i.e. those which had widespread RFs, and/or had deep, visceral, or maxillary input, and/or did not project to thalamus, was histologically verified to lie in the most ventromedial LCN. This group may be an interomniotopic element.

A corresponding toposograpy has been observed in the contralateral projection of LCN neurons to ventrobasal thalamus with the horseradish peroxidase (HRP) technique. Cells in the lateral LCN (LCN lateral) 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 were labeled from callosal parts of VPL, and from VM. This result is consistent with the physiological identification of a special subpopulation of neurons in the LCN. These results may provide further indication in the organization of the spinocervicalthalamocortical pathway, analogous to that of the dorsal column system, albeit with less discriminable projection in its counterpart. The above observations were further underscored by the identification of a special subpopulation of LCN neurons, which is separable from the main population of projecting neurons and which projects to somatosensory areas of the cortex.

In the present investigation the technique of retrograde axonal transport of horseradish peroxidase (HRP) has been used to identify the cells which send projections to the cervical segments of the spinal cord. LCN neurons were stained using HRP injections into the cervical cord. Stained LCN neurons were also detected in the contralateral spinal cord with HRP injections into the cervical cord. Stained LCN neurons were also detected in the contralateral spinal cord with HRP injections into the cervical cord. The results of these studies are consistent with the hypothesis that the cervical LCN neurons are involved in the control of motor function.
EFFECTS OF L-DOPA ON DORSAL HORN UNIT RESPONSES TO MECHANICAL STIMULI. Jonathan Delatriage, Charles J. Hodg C and Charles L. Woods*. Dept. of Neuromuscular, Upstate Medical Center, Syracuse, NY 13210.

Studies of the effects of L-Dopa on sensory transmission have suggested an inhibitory role for noradrenaline (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 threshold following completion of NA and a decrease in analgesia with increased NA. (eg Aki I & Liebekind, Brain Research 94:279, 1975). Thus there is evidence that NA has both inhibitory and facilitatory effects on sensory transmission. The 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 nociceptive unit responses in mammalian spinal cord. The effects of L-Dopa on nociceptive and non-nociceptive unit responses in the spinal cord of rats and mice showed that the effects of L-Dopa on nociceptive transmission were long-lasting and involved in the spinal cord. (Supported by NS grant NS 15361-01)


The broad sensitivity of individual neurons to several stimulus parameters in somesthesic responses is problematic in explaining the precision of tactile localization and discrimination. This was evident to Adrian (1931) who showed that individual somesthesic responses in the frog skin range from 4-100 mm/s and to Tower (1940) who observed that RFS on the cornea and scula of the rat range from 50-200 cm/s. Many investigators, using suprathreshold stimuli, have shown that the total number of RFS that are large relative to tactile discrimimability. Adrian hypothesized that stimulus location is signaled by the "particular combination of activity in action" and that the intensity of the discharge in each; a similar coding mechanism was suggested by Tower. Their similar positional theories have been largely undermined by those modern theories 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 somesthesic neurons are analogous to the problems posed in other sensory systems with broad-tuned neurons. However, a neuronal population response model, consistent with the ideas of Adrian and Tower, provides a solution. Application of the model suggests that the information of the thalamocortical and intravenous inputs, position, sound localization, temperature, color, etc. can be signaled by the profile of impulse frequencies in a responding population of neurons which is optimally large relative to the discharge of individual neurons per se. Intensity can be signaled by the total discharge frequency in the responding neuron population; the total discharge frequency rather than the RFS which are similar in quality produce similar AFPs.

Preliminary studies on the somesthesic system of the rat (Cashel, Anu; unpublished observations) have shown that the RFS model is applicable to the problem of tactile localization. Analysis in terms of RFS suggests that localization on the skin surface exceeds that in the forelimb and paws. It is seen that in this analysis, innervation density (neurons/cm²) is more relevant than that size. The breadth and complexities of the RFS, which may be troublesome as well as 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)

NON-SPECIFIC THALAMIC PROJECTIONS TO SENSORY-MOTOR CORTEX. John F. Donoghue and Ford F. Ebner. Neurosciences Section, Brown University, Providence, RI 02912.

The central intralaminar nucleus (CIN) of the opossum (B. virginiana) thalamus is located dorsal and medial to the ventrobasal complex. CIN receives input from all spinothalamic, coroid, and collicular regions (Walshe and Ebner, '73). Lesions of CIN result in a widespread projection from this nucleus to layer I of the somatosensory (S1) cortex (Killackey and Ebner, '72; '73). The present results demonstrate several additional features of these non-specific thalamic projections. Following a section of horseradish peroxidase (HRP) into physiologically defined zones of S1 cortex, retrogradely labelled neurons are present in CIN as well as in the ventral nucleus (VB). Injections of HRP into hand area leads to labelling in more lateral parts of CIN, while injections in the face area label more medial cortex. 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 latency of 60-120 ms to a variable latency after electrical stimulation of the contralateral body surface. They fail to respond consistently at remote points. Injections of HRP into the HRP topography. EM autoradiography and degeneration procedures reveal CIN terminals in the same subdivisions of layer I, with very rare and sparse degeneration in other layers. CIN terminals contain round synaptic vesicles and are associated with an asymmetrical or focal contact. Approximately 80% of these synapses contact dendritic spines. These studies demonstrate the properties of one type of non-specific thalamic neurones that are capable of projecting from the "specific" VB projection. Based on the experiments, CIN appears comparable to some of the locust projections in other mammals. (Supported by NSF grant NS 115361-01)

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. Stimulation 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 mediodorsal 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 bands 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 parallel relay pathways for different submodalities. 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)

COLLATERAL AFFERENTS TO THE INFERIOR OLIVE AND SUPERIOR COLLICULUS ORIGINATING IN THE SPINAL TRIGEMINAL NUCLEUS. Anthony Frankfurter, John Persing, and Oswald Stewart, Dept. of Neurosurgery and Physiology, Univ. of Virginia School of Medicine, Charlottesville, VA.

Horseshadid 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, 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 labelled neurons 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 that the extremely large number of labelled neurons in the contralateral interopolar division of the spinal trigeminal nucleus, a lesser number of labelled 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 labelled cells in the interopolar 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 dorsoventrally across the midline and rostrally. The other bundle can be traced exiting from the medial aspect of the cell group and coursing ventromedially across the midline and rostrally. These bundles are traced to the ipsilateral inferior olive, and terminating within the contralateral principal and dorsal accessory nuclei. A smaller contingent of trigeminothalamic fibers can be traced to the caudal levels of the inferior olive, where they appear to terminate within a mediodorsal segment of the mediodorsal 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 trigeminothalamic bundle. Although these fibers could not be traced in entirety, terminal label and axon fragments are present in laterally located cells in caudal portions of the contralateral accessory nucleus. The inferior olive is also an important input source to receive dorsal column afferents. In summary, this preliminary evidence suggests that the same neurons which provide the cerebellum, via the thalamic input, are also an important input to the somatosensory map contained within the deep layers of the superior colliculus.

DEGENERATIVE CHANGES IN DENDRITES OF INTERNEURONS IN LAYERS II AND III OF THE DORSAL HORN OF THE HERRULA IN DEVELOPMENTAL STUDIES IN NEONATE RATS AND FOLLOWING PULPITOMIES IN ADULT CATS. William Fails and Stephen Gobel. Neurobiology and Anesthesiology Branch, NIMH, Bethesda, MD. 20014

Most of the small, non-primary afferent terminals entering the substantia gelatiosa of Rolando in the caudal medulla synapse in layers II and III on dendrites of two major kinds of interneurons, small spiny stellate and small spiny round. These dendrites radiate in all directions from the cell bodies of the most immature forms of both of these cells. During maturation, a portion of these dendrites continue to elongate while the other branch up and disintegrate. Within the beads, small vesicles collect in aggregates and begin fusing with each other to form small clefts. The clefts continue to open 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. Degeneration of beaded dendrites takes place despite synaptically input from non-primary afferents. However, beaded dendrites have never been observed on a neuron with 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., 1977, 132(1977),347-354) and transynaptic 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 begin to form in the densest areas of the cellular plate 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 primary sensory dendrites is not sufficient to assure their continued elongation during development nor are they sufficient to assure their survival in the adult should their primary 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.

BODY SOMATOSENSOTY IN THE SECOND SOMATIC SENSORY CORTEX OF THE MONKEY. D. P. Friedman (UNIV. OF ILLINOIS). 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. Anatomical labeling procedures 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 were labeled with the labeling pattern of bands within SI. In the case of the forelimb area the label ran from posterolateral to anteromedial across an extensive region of the frontaloparietal operculum. This indicates that the face is represented anterolaterally and the hindlimb posteroomedial 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 horseshadid peroxidase into SI which retrogradely labelled cells in SII tended to confirm this pattern for the reciprocal projections in SI to SII.

The larger bands of label seen in the autoradiographic experiments were composed of smaller columns approximately 0.25 to 0.5 mm wide. The smaller columns in SII had a diameter of about 0.5 mm. The fact that SI is larger than SII and that its constituent fields (areas 3b, 4a, and 4b) are in a row rather than in a chain indicates that SII projections converge onto specific areas of SI.

Contrary to previous reports, no projection could be traced from the motor cortex to SII, though the projection from SI to motor cortex was confirmed passing from SII to the motor cortex.

(Supported by Grants No. NS 10526 and No. F32-NS00586 from the National Institutes of Health, U.S. Public Health Service.)
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.

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 (<60/sec) or slowly (<30/sec 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 Sensitivity 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/sec) triangular displacement of a single innervated hair was increased for longer periods (eg to 25 msec) following one or a few of electrically evoked antidromic spikes (cf analogous changes in velocity threshold following an antidromnic spike: ref.3). No sign of such long remaining 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.

(3) Tuckett, Horn, & Burgess (1978), J. Neurophysiol. 41:138-149
(Aided by USPHS, NIH Grants NS11219 and 10907.)


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 suited to this task. We have studied the ability of the QA population in the glabrous skin of the monkey to represent a simple spatial pattern containing 2 x 2 points vibrating at 30 Hz. This representation was 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 plateaus, as for single vibrating probes. Indicators of fiber sensitivity. Thus for our stimulus a QA's response was determined by a fiber-dependent 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 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. However, at a frequency of 100 Hz, 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 sensitivities of the QA population. Population responses were compared for vibrating stimuli and vibrating stimuli scanned across the skin such as occur with the Optacon.

The results show that the QA population is capable of representing spatial information used by blind people reading with the Optacon.

1 A prosthetic reading aid for the blind

562

In a previous quantitative and qualitative study of DG metabolism at vibrissal - cortical barrel system (Neurosci. Abstr., 3, 1977) confirmed the topographical organization of the SI barrel field and demonstrated the one-to-one vibrissal-barrel relationship. The laminar distribution of DG 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 DG labeling in normal and developmentally altered cortical column #3, row C in unanesthetized restrained adult raccoons. All vibrissae excepting #3, row C were clamped actively. Vibrissa #3 was stroked 2-3/s for 35-50 minutes, using a hand-held brush, to increase activity and uptake of DG in vibrissal orOF cortex 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 DG 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-III=8.4; II-III-98.1; IV=143.8; V=107.2; VI-93.8; and upper VI-73.3. The LCMRG for laminae of the barrel field, which surround column #3, row C were as follows: I-III-71; II-III-72; IV=197.2; V=167.2; VI-56.3. The normal laminar distribution of DG labeling in column #3, row C of the adult rat was significantly altered by vibrissal follicle-up (V-F) #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, fan-like pattern of labeling was no longer evident in lamina I-III or V-VI. Their labeling appeared diffuse; extending over a wide area of the surrounding barrel field. Thus, the paracortical (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.)


Single C-afferents, recorded with HCl-filled micropipettes in L4 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 decreasing excitation of single fibers. The stimulating electrode was moved in a stepwise manner down the column, and the 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 increment of 7.5 μs. The threshold for minimization of the stimulating electrodes, were 4-270 μA (median 66 μA) at 330 μs. The 78 units studied were either classified as high threshold (mechanoreceptors) or low threshold (15%), or C-mechanoreceptors (28%), or else they possessed no identifiable receptive field (28%). Although all units had increased thresholds after being orthodromically active, the time course of this effect (4 min. to recovery after orthodromic electrical stimulation of 30 Hz for 10 s.) resembled that of the hypoperfusion due to sodium pilling 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 increase in threshold occurred as follows: Noxious mechanical stimuli, I-IV and R-38; innocuous mechanical stimuli (brushing), I-III and R-32; moderate heat, I-III and R-38; noxious cold, I-III and R-18. Noxious cold to a single class unit, of the exact site of the conditioning stimulus, or of the 3 kinds of preparation (low spinal/decrebrate, decerebrate, and decorticate) with chloralose anesthesia was discussed. It seems therefore that more than one class of primary afferent can affect the excitability of C-fibers' terminals.

Orthodromic (2 mg/kg) or saline (2 ml/kg) injections did not change thresholds in 10 units. However, this does not preclude opiates having some presynaptic action, not affecting excitability, which influences post-axonal responses.

Trains of electrical pulses (20-50 Hz, 100-200 μA) in nucleus raphe magnus or adjacent reticular formation in 25 units gave 3-10 mV, in both chloralose-unanesthetized or chloralose-anesthetized, temporary block of the thornic spinal cord produced R-2, I-2, and 8 without effect. Thus descending influences are either tonic and excitatory and presynaptic to different brain regions, or not tonic and actively and mainly causing decreased excitability.

We conclude that terminals of C-fibers are modifyable by both segmental and descending influences.


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 SII and SII. The presence of interhemispheric projections to those areas 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 SII which are devoid of interhemispheric projections.

Injections of horseradish peroxidase and tritiated adenine 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 SII are located in layers three and five of the forepaw area in SII. The forepaw area in SII did send efferents nor did it receive efferents from the contralateral SI and SII.

2. The hindpaw area of SII is reciprocally connected with the ipsilateral hindpaw area of SII. The origin and termination regions of these reciprocal connections were located in layers three and five of the hindpaw area in SII. The cell bodies in the hindpaw area which sends efferents to the contralateral SII area are located primarily in layers three and five. The ipsilateral hindpaw are also 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 homotopic functional areas within SI and SII.

(Supported by NSF Research Grant GB 45236 and the Neuroscience Program.)
1777 RECORDINGS FROM CAT SPINAL CORD NEURONES WITH THALAMIC CONNECTIONS. James A. Holloway, Richard E. Fox*, Ainsley Jepson* and Subhilti S. Waks*. Department of NSR, also Physiology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH. U.K.

Adult cats were anesthetized with α-chloralose, subsequently paralyzed with gallamine triethiodide, and artificially ventilated. Following a lumbar laminectomy, glass microelectrodes containing 3M KC1 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 stainless steel electrodes, for diadic electrical stimulation (150μA, 0.3 msec duration) of spinal cord cells, was stereotaxically placed in the following contralateral thalamic nuclei: ventrals lateralis, ventrals posteromedialis, and centrum medianum. These nuclei are thought to receive spinthalamic tract axon terminals. Quantitative analysis of 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 foramina were observed in the dorsal horn in laminae-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; units excited post-synaptically from the thalami were from spinal cord laminae VI and VII. Post-synaptically excitable receptive fields were by far the most frequent recorded in response to thalamic stimulation. It is likely that at least part of this post-synaptic information is mediated via excitatory thalamo-neocortical neurones.

The results emphasize the diversity of response properties in some spinal cord neurones.


Supported by grants: SRC G/RA/2136.8 and NSR Award IF 34 GM 6519-01.NIGMS.


During the first few days of life, the pattern of the trignemohalmatic neurons that project to the thalamus 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 distributed in a pattern which replicates the topographical organization of the myotaxial vibrissae on the snout of the rat (Belford, Anat. Rec., 1978). Further, the cortical and thalamic relay cells of the rat 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 whether a 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 trignemohalmatic system.

In the present experiment, one "early" group of rats received parietal cortex injections of horseradish peroxidase on Day 0 (day of birth), and two "late" groups were given the development of the discrete afferent segmentations. A second, "late", group received similar injections on postnatal Days 5 and 7, after the development of afferent segmentations. After survival of 4 to 24 hours, the animals were perfused and the brains processed according to the technique of Mesulam (J. Hist. Cyto., 1976).

These experiments reveal that the distribution of labeling in thalamicnevrons in the ventrobasal complex differs markedly between the early and late groups. In the early group of animals, labeled neurones do not appear to be segregated into clusters, and the visible dendrites of labeled neurones can be seen radiating from the cells in all directions. However, although the neurones appear to be uniformly distributed throughout the ventrobasal complex on Day 0, it has some tendency for these neurones to form rows by Day 1. This corresponds well to the observed trignemohalmatic pattern at birth. In the older group of animals, labeled neurones are ever more closely related to the distribution of thalamicneurons. Neurones are segregated into rows with distinct row boundaries, and the dendrites of labeled neurones are orientated inward, toward the center of a row, thus respecting the row boundaries. Further, there is a noticeable tendency toward clustering of neurones within a row, although row boundaries are not as clear as those between rows. Thus a preferential sequence can be detected in the thalamocortical relay neurones of the ventrobasal complex, and this is the time of the afferent segmentations to the nucleus. (Supported by NIMH MH 14599-02 and NSF GB 41294.)

1779 FUNCTIONAL ORGANIZATION OF NEURONES IN AREA 2 OF MONKEY SOMATOSENSORY CORTEX/II OF Yoshikai Iwamura, Michio Tanaka* and Okihide Nikosaka* Dept. Physiol., Toho Univ. Sch. Med., Otaku, Tokyo, Japan.

The receptive field (RF) properties of the neurones 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 those units were trained to permit hand manipulation during experiments. The cortical unit recording was done with glass-coated platinum-iridium micro-electrodes. Neurones classified as "lateralized" were selected according to their RF characteristics: 1) simple skin units, 2) complicated skin units, 3) units responding specifically to joint manipulation, 4) units stimulated by RF 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 narrowing of the stimulated area was most important: they were activated most remarkably when certain elongated series of objects were passively contacted on the monkey's hand. Many units with defined skin units responded to joint manipulation. The most adequate stimulus for this type and some other non-skin units were often the complex patterns of stimuli produced by certain natural movements of the 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 neurones were compared with each other, it was found that a set of neurones with similar skin RFs were arranged vertically and that the largest RFs included other smaller ones as a subcategory. In the vertical organization non-skin units as well as skin and deep units were intermingled. It is suggested that the hand and finger region of the area 2 of the monkey's somatosensory cortex is a simple somatosensory but in terms of multiple clumping of neurones 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.


The anterior ventral (V) area of the owl is uniquely anatomically and physiologically similar to the striate cortex of mammals (Karten, et al., 1973; Pettigrew and Koniishi, 1976). In contrast, the smaller quadrangular posterior ventral (Vp) area of the owl is distinctly different from the anterior, being much larger, and having a much finer cellular structure. The owl's anterior ventral cortex is divided into an anterior wall and a posterior wall, each of which has a subcallosal region. Opaque structures in the posterior wall of the anterior ventral area are the primary somatosensory representation. The owl's pattern of receptive fields is similar to that of the mammalian primary sensory representation, including a topographical organization of somatosensory modalities along the vertical axis of the brain, with a somatotopic representation near the posterior wall of the anterior ventral area. The owl's V and Vp areas have a central representation of the somatosensory system, including the somatosensory representation of the head, neck, and body, including the ears, nose, tongue, body, and whiskers. The owl's V and Vp areas have a topographical organization of somatosensory modalities, including a somatotopic representation of the head, neck, and body, including the ears, nose, tongue, body, and whiskers. The owl's V and Vp areas have a central representation of the somatosensory system, including the somatosensory representation of the head, neck, and body, including the ears, nose, tongue, body, and whiskers. The owl's V and Vp areas have a topographical organization of somatosensory modalities, including a somatotopic representation of the head, neck, and body, including the ears, nose, tongue, body, and whiskers.
Seventy-eight elasmobranch fibers were recorded from the dorsal roots of deerebrate, curarized and artificially ventilated stingrays using 4 M NaCl glass electrodes with resistances of 25–35 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, our preliminary survey are many which respond to proprioceptive stimuli. These units are characterized by a very regular discharge pattern that is modified by elongation 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 thresholds 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 Polomorphidinoff ending reported by others.

This work supported by NIH grants NS 11255, NIH postdoctoral fellowships NS 05434 (to K.B.L.) and NS 05686 (to D.R.K.), and by a grant from the Muscular Dystrophy Foundation of America.

1782


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 30X 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 Hanks-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 Olzewski, 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, RO5333, and DE0001.)

1783

MULTIPLE UNIT ANALYSIS OF CUTANEOUS RECEPTORS USING PHASED BINARY CORRELATION. P. J. LOOT and M. S. FULLER. Bioclinical Sciences Laboratory, University of Michigan, Ann Arbor, MI 48109

Binary, phased correlation (Hoedters W. and Williams W., Science 186:373, 1975) was used to separate single units from population responses in peripheral cutaneous nerves.

Nerves were exposed on the inner surface of the cat's ear, as measured with the potent local anaesthetics, 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 with forceps and placed on silver electrodes in a mineral oil pool. Electrode separation was between 4 and 6 cm. A punctate stimulator was placed on the skin at a constant velocity in a raster scan covering 1 to 4 square cm. Stimulus force was 1 g.

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 (Loe, Amer. Zool., in press). (Lamina I-S, 1975-1976, unpublished).

Field, type I, and type II receptors produced similar topographic maps using single or multiple unit analysis (fig.2).

Further work is necessary to determine the cellular and neural mechanism corresponding to the analysis of complex spatial-temporal stimuli coding in peripheral cutaneous nerves. (Supported by USPHS grant NS 08470)

1784

AFFERENT PROJECTION TO THE NUCLEUS CENTRALIS LATERALIS IN THE CAT AS DEMONSTRATED BY RETROGRADE TRANSPORT OF HORSE-RADISH PEROXIDASE. Charlotte M. McDonald* and George M. Krauthamer (Spon: S. Rosner). Dept. of Anatomy, CMRI-Rutgers Medical School, Piscataway, New Jersey 08854.

As part of a series of experiments to define the anatomical connections of the intralaminar thalamic of the cat, horseradish peroxidase (HRP) was injected into the nucleus Centrals Lateralis (CL) and the brain examined for retrogradely labeled neurons. Electrophoretic injections through glass micropipettes were made 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 intermedio and the stratum griseum profundum; in the dorsal medial nucleus of the raphes; and bilaterally in the cuneiform 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 notably 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)

It is known that the dynamic properties of somatosensory cerebral cortical neurons are altered by the administration of central depressants. 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 cortex performed in the presence of general anesthetics are routinely regarded as providing a relatively undistorted view of the cortical representation of the body surface. The purpose of this study was to examine the effects of several commonly employed general anesthetics on the static properties of S-I neurons. Neurons in both rat and gerbil cortex were studied after a recovery from i.v. injection of the general anesthetics. For neurons in cytoarchitectural areas I and II, general anesthetics led to: (i) progressive reduction in the size of RFs; (ii) proximal and distal attenuation with progressive increments in anesthetic dosages; and 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 face 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 more digits. The RFs being more susceptible to different regions 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 RF being more susceptible to different conditions. For both cutaneous and deep neurons located in cytoarchitectural areas II and III the administration of general anesthetics in dorsal or ventral foramen for 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 caution in use of data obtained under this condition for the reconstruction of the cortical representation of somatosensory stimuli events. Supported by NS10865, DE2668, RR05333 and DE00011.

HRF MAPPING OF THE TRIGEMINAL GANGLION IN EMBRYONIC AND HATCHLING CHICKS. Siu W. Hui1, John E. Huguenard* (SPOM), S. A. George2, Dept. of Zoology, Univ. of Massachusetts, Amherst, MA. We have examined the innervation of the maxillo-mandibular but not the ophthalmic lobe varies depending upon the site of righting (caliber to the extent that these microelectrode penetrations are satisfactory). Application of HRP to areas of maximal nerve innervation results in labeling of many labeled cells scattered throughout the TG. In some cases neurons in the trigeminal motor nucleus are also labeled, indicating that damaged cortex. The labeling includes the TG. Application of HRP to areas of maximal nerve innervation results in labeling of a few core cells. In the hatchling injections of HRP into the TG are localized to the TG. The location of these cells correspond to the region of the TG from which axons to the injection site emerge, and is similar to that seen in the embryo. Labeled cells are always intermixed. The injection of HRP into the TG of newborn rats results in labeling of twice as many neurons but are more scattered. HRP is not preoccupation in the location of the TG devoid of neurons with cutaneous projections. These data prove that there is a patterned arrangement of TG neurons which is consistent with the pattern of peripheral fiber distribution but not with embryonic origin or neural circuitry.
ON VS. OFF RESPONSES OF RACCOON GLABROUS SKIN RAPIDLY ADAPTING CUTANEOUS MECHANOSENSORY. Benjamin H. Pubols Jr., Dept. of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA.

The mammalian rapidly adapting (RA) cutaneous mechanosensory is a class of nerve fibers that are defined by their response to a stimulus, as 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 (on-response), and a silence during b, and usually a discharge during c (the off-response). During d, the unit is silent. A frequent finding in previous studies has been that the response duration has been more vigorous than that during c. However, where specified, c = 0, and b = 0, and may thus be due to the fact that pre-ramp time has been less preceding off-ramp than on-ramp, 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 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 remainder, 15%, the off-discharge is absent.

2. On off indentation thresholds are approximately equal (3% median = 45); 54% median = 4).

3. On velocity thresholds are significantly lower than off-velocity thresholds (on median = 1 u/msec; off median = 4.5 u/msec).

4. Opponents of the model, however, suggest that such a difference in the sensitivity of these different stimulus sites may be differential encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the discharge of single mechanosensitive nerve fibers innervating the skin in terms of this model. Recordings were made from single primary mecanosensitive 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 footpad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The response field (RF) of each fiber responding to at least one test site was mapped at a standard set 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, individual neurons cannot unequivocally discriminate between changes in stimulus location, intensity, and quality. However, a number of population properties of the model can be used to show how the various stimulus parameters may be differentially encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the discharge of single mechanosensitive nerve fibers innervating the skin in terms of this model. Recordings were made from single primary mecanosensitive 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 footpad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The response field (RF) of each fiber responding to at least one test site was mapped at a standard set 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, individual neurons cannot unequivocally discriminate between changes in stimulus location, intensity, and quality. However, a number of population properties of the model can be used to show how the various stimulus parameters may be differentially encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the discharge of single mechanosensitive nerve fibers innervating the skin in terms of this model. Recordings were made from single primary mecanosensitive 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 footpad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The response field (RF) of each fiber responding to at least one test site was mapped at a standard set 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, individual neurons cannot unequivocally discriminate between changes in stimulus location, intensity, and quality. However, a number of population properties of the model can be used to show how the various stimulus parameters may be differentially encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the discharge of single mechanosensitive nerve fibers innervating 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 footpad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The response field (RF) of each fiber responding to at least one test site was mapped at a standard set 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, individual neurons cannot unequivocally discriminate between changes in stimulus location, intensity, and quality. However, a number of population properties of the model can be used to show how the various stimulus parameters may be differentially encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the discharge of single mechanosensitive nerve fibers innervating 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 footpad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The response field (RF) of each fiber responding to at least one test site was mapped at a standard set 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, individual neurons cannot unequivocally discriminate between changes in stimulus location, intensity, and quality. However, a number of population properties of the model can be used to show how the various stimulus parameters may be differentially encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the discharge of single mechanosensitive nerve fibers innervating 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 footpad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The response field (RF) of each fiber responding to at least one test site was mapped at a standard set of stimulus intensities; discharge characteristics at different stimulus sites and intensities were examined. 
1793 MODULATION OF SOMATOSENSORY CORTICAL NEURONS BY CHANGES IN BEHAVIORAL AROUSAL AND ATTENTION IN THE RHESUS MONKEY. J.R. Roppolo and J.J. Collins. Dept. of Pharmacology, Sch. of Med. U. of Pittsburgh, Pittsburgh, PA 15261

Previous studies from our laboratory (Fed.Proc.26:471, 1977) have shown that alcohol and other central excitatory drugs cause marked changes in arousal and attention can profoundly alter spontaneous activity (spont.act.) and stimulus representation in the primary somatosensory cortex (SWS) (1-4). The purpose of this 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 the SWS of alert, non-anaesthetized monkeys. Peripheral receptive fields, located on the glabrous skin of the hand, were stimulated by a fine brush moved back and forth across the receptive surface in a consistent manner. Each subject was placed in three behavioral states: (1) awake state-in which the monkey simply sat quietly in a sound attenuating recording chamber with both the experimenter and the recording equipment in the room; (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 visually cued sustained behavioral task. The task involved discriminating between two sets of stimuli: white light in the left eye and green light in the right eye, presented visually for either 200 or 500 msec intervals presented in a random sequence. The white light stimulus was indicated by the experimenter and the green light by the subject. When the subject responded correctly, a 150 msec duration reward was given. The brushing stimulus was presented every 5 sec., but provided no appreciable increase in behavior.

1794 PATTERN OF AFFERENT SUPPLY TO CANINE SPINAL TRIGEMINAL NUCLEUS. J.J. Schneider and A.L. Itatli. Division of Neurosurgery, Dept. of Surgery, University of Pittsburgh, U.S. Naval Hospital, Baltimore, Maryland 21201

Electrical activity was recorded from the branches of the trigeminal ganglion in dogs. Wire electrodes were insulated in similar fashion as the branches emerging from the ganglion, and placed in two fossa for acute recording or left in place during recovery and used for chronic recording. A conventional electrophysiological system was used for recording. In the present experiment the goal of these experiments was to define the boundaries of the ophthalmic, maxillary and mandibular receptive fields in dogs.

Previous studies (1) have documented the presence of the maxillary and mandibular branches that could not be differentiated by the level at which several cranial skin regions terminated. This contradicted results which reported that the maxillary and mandibular branches were not separated in the trigeminal ganglion and therefore there were no separate maxillary and mandibular fibers ending progressively less caudal, respectively. An opposing notion, of the terminations being cell-specific, was also made. The present experiments demonstrated the existence of patients with sensory losses due to syringomyelia.

In humans, this trigeminal region is centered around the nose tip. The first two trigeminal branches only have a cutaneous representation, but the third branch extends to the upper lip and connects to the maxillary division of the trigeminal branch. To determine if these hypothesises were present, we had to define the receptive fields of each branch of the trigeminal ganglion. This is the object of this study. This task was in order evidence for either maxillary or mandibular-like segregation within the canine spinal trigeminal nuclei.


Recordings were made from fine filaments dissected from the great auricular nerve. When an isolated action potential could be recorded at C velocity was evoked by electrical stimulation of the nerve, a systematic effort was made to characterize its sensory properties. In the present study, we examined the responsiveness of sensory units to thermal stimuli in the human hand. In the present study, we examined the responsiveness of sensory units to thermal stimuli in the human hand. Changes in tactile and temperature sensations were observed in 4/5 of the test site but were not observed in the number of cases.


Neurons in pericuricate cortex (field 4v) 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 the bladder or rectum. Some neurons respond to somatic and visceral input. The present study examined the influence of pudendal nerve stimulation on pericuricate neurons. The pericuricate nerve (CN II) is a fascicle of the pudendal nerve which innervates the penis and the other structures. The pudendal nerve was exposed dorsally, placed on hook electrodes, and covered with petrol. A bipolar stimulating electrode was placed across the pudendal nerve, the contralateral pudendal input serving as stimulus. Extracellular recordings were made of neurons in the contralateral forelimb cortex of the cat in a chloralose-anesthetized male cat. Neurons were classified as so if they responded only to one pudendal nerve, as sb if they responded both to pudendal nerves and both hindpaws, and as s if they responded to all four paws. Most m neurons (95%) were excited and the remainder were facilitated by pudendal nerve stimulation, whereas few ab (18%) or sa (3%) neurons were either excited or facilitated by this input. All as and ab neurons were facilitated the most by pudendal nerve stimulation and facilitated by stimulation of the nonexcitable paws — suggesting that they share some characteristics in common with m neurons. When tested, the contralateral pudendal nerve stimulation facilitated the activity of these neurons to pudendal nerve stimulation. That is, the position of any m neuron with respect to its fellows in the responding population remains unaltered by pudendal stimulation. Pudendal stimulation results in a broad range of responses among neurons that are responsive to pudendal nerve stimulation. The exact nature of these responses is not yet known. It is possible that the responses are due to changes in central synaptic activity or changes in the responsiveness of neurons to the specific inputs. The results of the present study suggest that the responses of neurons to pudendal stimulation are complex and may involve both central and peripheral mechanisms.

The data suggest that the responses of neurons to pudendal stimulation are complex and may involve both central and peripheral mechanisms. The results of the present study suggest that the responses of neurons to pudendal stimulation are complex and may involve both central and peripheral mechanisms. The results of the present study suggest that the responses of neurons to pudendal stimulation are complex and may involve both central and peripheral mechanisms.
STRUCTURAL AND FUNCTIONAL POPULATION PARAMETERS DETERMINING INPUT-OUTPUT RELATIONS AT THE CUNEATE.

J. P. Vahle and D. Whitehorn, Dept. of Physiol. and Biophys., Univ. of Vermont, Burlington, Vt. 05401

The evoked total impulse activity (TOTACT) within a neural population (CUN) of the leg, and the product of this activity in terms of the number of impulses per active cell (NIFACT), was determined at a number of intensities for each input-output relation (IOPC), while recording from individual neurons. In six animals, the evoked total impulse activity was determined at 10 intensities for each IOPC using 200-msec stimulation pulses. The total impulses (TAC) for each IOPC were determined by a computer program, the output of which was a histogram of impulses for each intensity. The number of impulses per active cell (NIFACT) for each intensity was determined by dividing the TAC by the number of active cells (NACT). Prior input-output (IOPC) studies using evoked potentials to measure output were only the 2nd relation between TOTACT and input. We have additionally acquired the 10 relations for NIFACT and NIFACT by recording from individual, identified neurons in CUN during cyclical stimulation of the compound action potential recorded from a second site on the nerve. The 10 relation for TOTACT, both rising steeply and displaying saturation, NIFACT increased only slightly with increasing input.

We conclude that the 10 relation for TOTACT is primarily determined by the recruitment of CUNC cells into activity (NIFACT) with changes in the number of impulses per active cell (NIFACT) having a minor role.

The 10 relations for TOTACT and NIFACT are well described by equation 1:

\[ y = \frac{1}{\lambda} \cdot \exp(\lambda x) \]

where \( y \) is fraction of output cells active and \( x \) is fraction of input cells active. The condition is consistent with a population model with moreafferents 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 neurons). As a result of the 10 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 nucleus.

Supported by NIMDD: NS059472.

ORGANIZATION OF THE IPSILATERAL AND CONTRALATERAL INPUT TO THE NEURORECEPTORS OF S-III/I IN UNANESTHETIZED MONKEY.


Responses of single neurons and neuron clusters were studied in the course of microelectrode penetrations of the anterior portion of the somatosensory receiving area (S-III/I). The effectiveness of hand-held or tail applied stimulation and simultaneously to bilaterally symmetrical cutaneous body regions was determined for a large population of S-III/I neurons. With the exception of the hand and tail, the only site which could be activated only by unilateral stimulation, most S-III/I neurons could be classified as follows: (i) dominated by contralateral input; (ii) dominated by ipsilateral input (iii) active 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 category. Penetrations across S-III/I 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 (occclusion) than that evoked by the most effective unilateral stimulation. It is anticipated that further aspects of the functional organization of bilateral input to area S-III/I will be revealed by plotting the distribution of each neuron class.

To date, a limited sample of S-III/I 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 or tail stimuli, these studies have revealed that the static and dynamic properties of the responses evoked from a single S-III/I neuron by separate stimulation of the ipsilateral and contralateral cutaneous field of its receptive field were "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 RO10686, RO24648, RO5333 and RO50011.

POSITION AND MOTION SENSE UNDER WHITE NOISE STIMULATION.

W. J. Williams and B. W. Roessner, Jr., Biophysical 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 motion and positioning the two types of sensation are entrained and difficult to separate experimentally. Preliminary experiments using very low frequency sinusoidal joint motion showed that the position motion was lost below 0.01 Hz. Position sense remained, but was discontinuous and new positions were suddenly perceived at a point where the joint jumps. 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 creating a simulated position and motion sense using band-limited (0-0.16) white noise.

The general protocol was similar to that previously reported (Kolmen, 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 the subjects responses for 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 is the product of the two sensations and declines precipitously for very low frequencies below about 0.01 Hz. Supported by U.S.F.R.S. Grant N0 68470.
SOCIETY FOR NEUROSCIENCE

SOMATOPTIC ORGANIZATION OF THE RAT CORTICOSPINAL AND CORTICO-
TRIGEMINAL SYSTEM. Steven F. Wise and Elizabeth A. Murray.
Marine Biomedical Institute, Galveston, Texas 77550.
The projection from the 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 diaminosidine 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 IV with the underlying dense, granule cell aggregates in layer V, since Wielker (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 105736).

VELOCITY DEPENDENCE OF SOMATOSENSORY NEURON RESPONSE TO MOVING TACTILE STIMULI. H. Young, R. Schreiner, B. Whistler, and D. Dreger. 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 R²=0.89). 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 R²=0.84) 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 tracks.

Supported by NS10865, DE2668, RR5533, MH14277 and DE00011.
SPINAL CORD
PERIPHERAL INPUTS TO LONG DESCENDING PROPSYMPATHETIC NEURONS IN CAT. Robert J. Adams, Robert D. Skinner, and Ronald S. Rempel. Dept. of Anatomy and Physiology-Biophysics, Univ. for Medical Sciences, Little Rock, AR 72201.

Long descending propssympathetic neurons located in the cervical cord project to the thoracic cord (Skinner et al., Neurosci. Abs. 3: 508, 1977). Cats anesthetized with ketamine were spinalized at C1, unemecally decerebrated, and the anesthetic discontinued. The cord at C1 was stimulated with antidromically excited cervical neurons, which were recorded from extracellulary with microelectrodes. Criteria for antidromicity: predominantly negative spikes with sharp leading hump, the ability to follow four to five spikes at above 300 Hz. Collision with orthodromically excited spikes was verified. In half of the experiments the intact peripheral nerves (superficial, deep radial, superficial radial, ulnar, median and musculocutaneous) were stimulated with cuff electrodes.

In segments C1-T6, 236 long descending propssympathetic neurons were recorded, with conducitonal 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 forelimb. The response field of these varied 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 joints in the forelimb. Responses to noxious touch, heat and noxious pinch; 23% to joint movement, usually from the metacarpophalangeal or interphalangeal joints; and 41% to hair movement. Of these, 91 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 3% to the whole body, representing the anterior half of the body. At 0.01 sec after the stimulus, the multivpeated unit potentials were recorded from the afferent volley to the contralateral hand, at 750 Hz. The responding field was observed from the ipsilateral field in 10 cells and from the contralateral level 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 and 40 msec at the time of the afferent volley to the cord were: 6.0 ± 3.8 msec (mean and std. dev), which indicates a monosynaptic path. Only a few latencies were found, which is not to suggest a monosynaptic connection. (Supported by NIH Grant RR05550 from NCRM.)

THE IA EPSP IN MAN. Peter Asby, Duane Zill AS, 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 muscle and identifying each occurrence of a given motor unit action potential. Alternations in the firing of (as a result of afferent volleys) were used to deduce the underlying post-synaptic potentials produced in that neuron.

Homonymous group 1 volley was produced an early peak of increased impulse density in the post-stimulus time histogram (PSTH) of a rhythmically firing human tibialis motoneurons which was of appropriate latency to represent the IA EPSP. We characterized the properties of this EPSP in 4 stages:

1. The time of the IA 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 IA EPSP was determined by injecting a single volley and measuring the increase in the membrane potential. 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 12 mV less, the amplitude of the EPSP can be obtained by simple geometry.

3. The duration of the falling phase of the IA EPSP was determined by detecting 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 determine the characteristics of post-synaptic potentials in man. Monophasic trains can be recorded from CNS neurons in man, the method is not restricted to the study of spinal motoneurons.

THANK the Conner Foundation; Computer facilities were made available by the USAF; AFOSR 75-2804.


The effects of phentoin (PHT) and chlorpropramide (CPZ) on spinal cord monosynaptic discharges and post-tetanic potentiation (PTP) were compared in groups of decerebrated anesthetized cats: (1) cats with high spinal section and (2) intact cats. PHT produced no significant change in the monosynaptic discharge of either 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 min. 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 were 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 firing fringes in the spinal cord, (2) CPZ depresses descending excitatory influences on the spinal cord without interfering with recruitment, (3) the effects of PHT + CPZ 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 stimulation between low and high frequencies preventing 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 NIA Foundation.)


Monoamine oxidase (MAO) activity was measured in microdisected samples of central grey matter (CM) and surrounding white matter (WM) from canine spinal cord at various intervals following 400 gram trauma. Both C(6)7-aminoxylic (SHT) and C(1)4-tranylcypromine (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 trauma.

Activities obtained, in terms of moles deaminated product formed/15 min, resulted not for non-traumatized controls were summarized in 4 stages, where

\[ \text{SHT} \quad \frac{0.00414\pm 0.0002}{0.118} \quad 0.1344 \pm 0.017 \]

\[ \text{TRY} \quad 0.1344 \pm 0.018(4) \quad 0.1344 \pm 0.017(4) \quad 0.1344 \pm 0.017(4) \]

MOA in traumatized CM dropped significantly (50%) from control levels, with SHT as substrate, as early as 15 minutes post-trauma and remained so through one hour post-trauma. TRY deaminating activity in CM was not significantly decreased until 30 min. - post-trauma (172) and showed a 23% 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 compared to non-traumatized control. TRY deaminating activity in CM was not significantly decreased until 30 min. - post-trauma (172) and showed a 23% 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 compared to non-traumatized control. TRY deaminating activity in CM was not significantly decreased until 30 min. - post-trauma (172) and showed a 23% decline (nearly 50% of control) at one hour post-trauma. TRY deaminating activity in CM was not significantly decreased until 30 min. - post-trauma (172) and showed a 23% decline (nearly 50% of control) at one hour post-trauma.

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 sparse or absent. The method of a modified serially impregnated axonal technique (Kumazawa and Perl, J. Comp. Neurol. 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 anisiform axonal complexes, previously described in the monkey (Beal and Fox, J. Comp. Neurol. 168: 111, 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.

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 afferent fibers to the spinal cord, the authors have modified the technique described by Prohaska (Neurosci. Lett., 5: 130, 1977) for the anterograde injury-filling of these axons and their terminals. For these experiments, the nerve roots were transected and filled with HRP for 401 solution of HRP for 12-18 hrs. Tissue was processed according to established histochemical procedures for the visualization of reaction products. 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 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-core vesicles. Terminals with dense-cored vesicles were confined to the ML and outer major portion of laminae I and II. The profiles 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 Golbe (J.C.N., 167: 165, 1976) in the SG of the spinocerebellar nucleus. Some terminals made synaptic contact with larger dendritic profiles located outside of the 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 Post-doctoral Fellowship NS-05394.)

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 R. Richard Koerber. Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Adult cats were anesthetized with urethane (1.1 g/kg), paralyzed with Pancuronium bromide, artificially respirated. The spinal cord was transected between T10 and T12. Pairs of cells were recorded simultaneously from independently manipulated stainless steel micro-electrodes. Recording sites were verified to be located in laminae I-VI of L5-S1 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 nonsynaptic excitatory connections (brief positive deviations at monosynaptic delays) were seen.

In the connectivities 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.


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 record 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 2.41-g/cm2 dorsal column autodynamics (DCS) technique to measure SCBF at 1 hr, and 6 hours after moderate (260 g/cm2) or severe (500 g/cm2) impact injury to the cat spinal cord at T6. We measured SCBF in 2 cats at each interval, for each trauma dose. Control means SCBF for cord 3 cm or more from the trauma center in the 500 g/cm2 group was 11.6 g blood/min/100 g tissue for white and 14.0 g/mg tissue for gray. These flows agreed with those obtained by Landan et al. in awake intacts (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 period were obtained. The SCBF at 1 hour after severe trauma was 83% at 1 hour, 87% at 4 hours, and returned to 75% at 8 hours. In this group, the flows at 2 mm away were similar to controls at the same time period. At 1 hour, 1 mm away we significantly greater (62% of uninjured). Longitudinal spread of ischemia and hemorrhagic necrosis were greater for severe than for moderate trauma. Also the terminology hypereynia was more commonly seen after moderate trauma. Above we report mean white matter SCBF, but this obscures differences between flows within the spinal cord. For example, at 1 hour after 500 g/cm2 trauma, one cat showed hyperemia (18.2 to 23.2) in ventral white and normal flow (12 to 14) in lateral and dorsal white; within 2 hours the cord and cauda equina were still hyperemic (18.1 to 25.9). The last 24 hours the severe 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 differences in flow. We also note that mean SCBFs may be 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 term deficits of spinal cord injury (500 g/cm2) may be due in part to the greater white matter ischemia near the site of severe trauma compared to moderate (260 g/cm2) 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 NS 10174 and NS 54009.
1811 AN ELECTRON MICROSCOPIC STUDY OF THE TRACT OF LISSAUER. Kyung Soon Chung, Arnold E. Applebaum, and Richard E. Coggeshall, Deps. of Anatomy and of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77550.

In 1985, Lissauuer described a tract of finely myelinated fibers that was located over the dorsal horn of the spinal cord. Lissauuer felt that the majority of fibers in this tract were primary afferents. In later years, however, this view has been superseded and it is now felt that the fibers in the tract of Lissauuer are predominantly propriospinal in nature. The primary evidence for this conclusion is that few fibers in the tract of Lissauuer appeared to be affected when the dorsal root was cut. There are 7 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 Lissauuer was reexamined, both in the normal rat and after dorsal root section at the T8 and L7 levels. Our first results are as follows:

<table>
<thead>
<tr>
<th>Normal Side</th>
<th>Operated Side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myel. Fibers</td>
<td>2715</td>
</tr>
<tr>
<td>Unmyel. Fibers</td>
<td>6850</td>
</tr>
<tr>
<td>Lissauuer</td>
<td>75</td>
</tr>
</tbody>
</table>

These figures suggest that 853 of the axons in the tract of Lissauuer are primary afferents. Similar findings have been obtained from an L7 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 Lissauuer are primary afferents.

In addition synaptic terminals were found in the tract of Lissauuer. At present we are characterizing the synaptic types. We are also examining the tract of Lissauuer 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 interneuron interactions which have recently been reported.

Supported by Grants NS 10161, 11255 and Fellowship NS 05430 from the NIH.


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 restricted to the single, isolated phenomenon occurring in the terminal sensory neurones facilitated by returning 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 experiments. Each individual was examined individually 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 evoked in terms of 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 surface. The essential procedure was to 1) establish baseline responsiveness to the stimulus in terms of EMG activity at 24 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 electromedical responsiveness. No effect was demonstrable, however, on the retention of habituated withdrawal activity in terms of either the decrease in stimulus responsive EMG activity or the 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 electromedical 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-56813-6/4).

1813 PROFILE OF MYELINATED AXONS IN THE LUMBAR VENTRAL ROOTLETS OF THE CAT. P. L. GILDEMBERG, K. G. CHUITMAN* 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 anesthetized with sodium pentobarbital and a lumbar laminectomy (L3-5) was carried out. After perfusion of the spinal cord with 10% formalin, the processed segments were impregnated with osmium tetroxide (Palade, 1952) for 30-48 hours. After dehydration, the roots were embedded in paraffin wax. Serial section at 5 μ 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 L5 to L1 in a caudal direction, the ratio of gamma to alpha increases with respect to the smaller alpha and gamma axons. While the occurrence of gamma axons is of the order of 30-40% in L5, L4 and L3, the smaller roots, the occurrence of gamma axons in L2 and L1 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


1814 GLUCOCORTICOID EFFECTS ON SPINAL CORD REPETITIVE MONOSYNAPTIC TRANSMISSION AND APPARENT TRANSMITTER TURNOVER. EINAR 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., in press. Abst. 3502 and J. Pharmacol. Exp. Ther., in press). The principal findings were an enhanced monosynaptic (2N) post-tetanic potentiation, an increased rate of 2N EMG recovery and spontaneous 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 2N afferent to terminate. Monosynaptic reflex 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 activity 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 la activation in the treated vs. 27.6% 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 the first 60 seconds. The 2N plateaus in the treated were 13.9% (p>0.01) higher at 5Hz and 17.0% (p>0.001) higher at 10Hz than in the untreated. Comparative analyses of the extent of decline in two aspects of receptor transmitter release parameters (Capek and Elpin, J. Neurophysiol. 40:95, 1977) revealed no significant effects on the apparent fractional recovery of transmitter (p) by the L2 terminals, but the rate of replenishment of the available transmitter store (r) was more than doubled in the treated preparations.

As the decrease in the actual amount of transmitter 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, the maintenance of the repetitive transmission at moderate frequencies reflects an increased rate of transmitter mobilization. These direct facilitatory effects on central terminal release partly explain the beneficial effects of glucocorticoids in acute spinal cord trauma and certain other central neurologic disorders.

(Supported by NIH grants 5-R01-MH-01447 and 5-S07-RR05396-15). Present address for E.H.: Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.
1815 CONVERGENCE OF CUTANEOUS, MUSCULAR, AND ARTICULAR INFORMATION ON SPINAL NEURONS IN THE CAT. Christopher H. Honda*, Hiromi Bya*, and Irving H. Waggan (SPON: E. Sassenrath). Department of Animal Physiology, University of California, Davis CA 95616.

The degree of convergence between the sural nerve (SN), the nerve to the medial head of the gastrocnemius m. (MGm), and the posterior articular nerve (PAN) onto single neurons was studied. Extracellular unitary responses were elicited in the ventral spinocord 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 potential.

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 A6 and C fibers. Very few units responded exclusively to high levels of stimulation. Histological verification of recording sites with iontophotographically applied pontamine sky blue showed that the convergent units lay in laminae I-VIII, the majority being located in VII and VIII.

Other units were characterized as responding only to SN and MGm (11%), SN and PAN (5.0%), MGm and PAN (1.7%), SN (20.2%) and PAN (2.3%). No units responded only to stimulation of MGm.

The receptive fields remaining after nerve section were mapped for many convergent units in response to natural stimulation. The resultant complex receptive fields are suggestive of an "unmasking" effect which reveals peripheral influences extending over several segments.

The great degree of convergence from different peripheral nerves and fiber diameters within each nerve reveals synaptically connected and local reflexive pathways may share common interneuronal pools. (Supported in part by NIH grant AM81576)


Pouch-young opossums ranging in size from 15-105mm crown-to-crown length were subjected to the Falck-Hillarp technique or to ptyoxylic acid perfusion and freeze drying (Loren et al., Histocmt., 49: 177-192, 1976). Observations were made on cervical, thoracic and lumbar 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 is 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 lumbar levels. The fluorescence increases progressively in the intermediate zone at all levels of the cord, and by 55m it is found in most of the areas expected from adult material. Such areas include: the dorsolateral intermediate zone, the periphery of the ventral horn and the region carrying the dorsal horn (presumptive lamina I). In the oldest animals studied (60-105mm) the pattern of varicosities is distinct with variable varicosities. Supported by U.S.P.H.S. Grants NS-07410 and NS-10165.


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 mcg/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 hindpaw. 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 S1 motoneuron nucleus where activity was observed 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 generated 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) 216:683, 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.


Recent evidence has delineated both spinal (Aneseth. 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 noxiception. 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 Kitabata et al. (Aneseth. 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.
1821 BRANCHING OF AXONS IN THE L, S, AND S DORSAL ROOTS OF THE RAT. Lauren A. Langford* and Richard E. Coggeshall. Dept. of Anatomy and Physiology and Biophysics, and The Marine Biological 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, S, or S dorsal roots of the rat than there were ganglion cell bodies in the corresponding dorsal root ganglia. The following 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. (Alternatively, it is possible that a number of post-ganglionic 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 that 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 gangliectomy, no normal myelinated fibers were found but approximately 300 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 sympathetectomy was performed on one rat, and the dorsal root was removed after allowing for degeneration of postganglionic fibers. In the one rat examined to date, there were 4757 dorsal root ganglion cells and 7427 dorsal root ganglion cells in the S dorsal root and 4725 dorsal root ganglion cells in the S dorsal root. If further studies support these preliminary observations, the conclusion would be that there are more dorsal root axons than ganglion cell bodies in the corresponding dorsal root ganglia. Variations, however, due to the dorsoventral gradient to cutaneous receptors are excluded. According to textbook descriptions that indicate that each dorsal root ganglion cell sends a single axon into spinal cord through the dorsal root. Supported by NIDH grants 1017 and 11255.


To identify the morphology of physiological cells in the spinal cord, we have studied the classical laminar and sublaminal zones of the spinal cord, which are primarily identified by the presence of certain cell types. In the present study, we examined the morphology of neurons in the lamina II of the spinal cord. We have identified these neurons by their unique morphological features, such as their preferential location in the superficial laminae, their orientation, and their size. In addition, we have used various staining techniques, such as fluorescent labeling, to identify these neurons. Our results indicate that these neurons are of a particular type, with a dendritic pattern that is characteristic of neurons in the lamina II. These results suggest that the morphology of these neurons is different from that of neurons in other laminae, and may provide insight into their function in the spinal cord. Supported by the Canadian Medical Research Council.
1823 EPSPs ELICITED BY SINGLE IMPULSES IN LARGE POPULATIONS OF MOTONEURONS. Hans-R. Lüscher, Eberhard Fetz, Paul Rauezl, and Theodor Hemmen. Dept. of Physiology, Harvard Medical School, Boston.

The number of terminals given off by an axon of a motoneuron in a function of its length (Hemmen, et al. Neurphysiol. 28:71, 1965). Furthermore, evidence has been presented that the amplitude of an individual stretch evoked EPSP in a motoneuron is related to the number of terminals given off. The present paper reports data as to the number of impulses (Mendell et al. J. Neurophysiol. 34:171, 1971). This suggests that the number of terminals from a single fiber to a motoneuron is a function of its diameter. It is uncertain whether this inference is correct, 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 inference could be confirmed by averaging the EPSPs by single impulses in a large number of motoneurons, the total synaptic effect of a single 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 input potentials from a single, functionally isolated but intact afferent, the synaptic effect of a single impulse upon the whole population of motoneurons can be examined 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 related to individual postsynaptic-potential potentials.

Individual EPSPs were recorded from S3 ventral roots of cats under chloralose anesthesia. The conduction velocity of the afferent impulses in originatig dorsal roots was determined, and impulses were applied to the range group for i group and group II afferents. The amplitude of these EPSPs showed a consistent and significant relation to the conduction velocity of the impulses in slowly conducting fibers. The fibers in slowly conducting fibers yielded smaller EPSPs, while fast fibers were associated with larger EPSPs. Group II impulses elicited very small EPSPs.

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 of the EPSP also applies to primary sensory neurons in the stretch reflex.

Supported by a grant from the National Institutes of Health.

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 Tischendorf. Dept. Anat., Sch. Med., The Ohio State Univ., Columbus, Ohio, 43210.

Injections of H-leucine were placed into each of the cell groups revealed by an anterograde technique in the cervical spinal cord of Opossums. This revealed a number of reticulospinal projections. The developed autoradiograms reveal that the nucleus cuneiformis projects by the ipsilateral subarachnoid and ventral to lamina VII (Sk-LI) and the lateral reticular nucleus (LI) bilaterally through dorsolateral tracts to lamina I. When injections involve specific neurons in the ventrolateral part of the reticular BP there are two populations which radiate via the nucleus cuneiformis and/or adjacent areas producing labelling within the ventral, lateral and dorsolateral white matter, ipsilaterally, as well as labelling of axons in the contralateral ventral funiculi bilaterally. Many of the contralateral axons course among rubral axons. Some of the sites with injections of the dorsolateral pons show light labelling within lamina I and lateral parts of laminae VI, VII, bilaterally, as well as within laminae VII and VIII (mainly ipsilaterally) and lamina X. Injections of the nucleus reticularis gigantocellularis pars ventralis (RCv) produce labelling within the ventral, lateral and dorsolateral white matter, bilaterally, as well as terminal label within lateral parts of laminae VI and VII, ipsilaterally, and laminae VII, laterally, bilaterally. If serotonin containing neurons of the RM and the RCv are included in the injection there is evidence of a strong projection to laminae V, VI, IX and X bilaterally. The pattern of spinospinal projection is produced by serotonin injections to RM. Injections which include paramedian areas of the medulla, as well as the nucleus cuneiformis, produce retrograde axonal labelling within both the ventral and lateral funiculi and terminal labelln within laminae VII through X. Label is especially heavy within laminae IX and X. Injections limited to the medulla result in spinospinal projection from the medulla also result in spinospinal labelling and which cover the rostral lateral reticular nucleus and labelling of terminal laminae which extend into the intermediolateral cell column at thoracic levels. The nucleus retroambigius 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.


Long-Evans hooded rats were cordotomized at the T5 level and given: 1) cyclophosphamide, an immunosuppressive agent; 2) PIROMIN, a bacterial polysaccharide-nucleic acid complex; 3) topical and subcutaneous trypsin; 4) no treatment. Because of past and present controversy surrounding the proposed ability of these agents to promote spinal cord regeneration, a systematic study was undertaken of the histological, autoradiographic, immunohistochemical, 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 connective tissue with occasional fibroblasts and macrophages, or a loose, areolar tissue with numerous sheets and cords of mesodermal cellular elements but minimal collagen. At 45-65 days postoperative (dpo), axons, supported by Schwann cells, invade and become entangled in the loose connective tissue matrix. With longer postoperative survival, axons appear cranial and caudal to the lesion and erode much of the scar together with viable neural tissue. Administration of Cytoxan and PIROMIN did not result in any quantitative alteration of the scar matrix as evidenced by electron microscopy. Quantitative analysis revealed a slight reduction in the fibrinous connective tissue component of the scar at 1 to 5 dpo as compared with an earlier study from this zone but this proved to be a transient occurrence as longer postoperative periods were studied. The use of trypsin resulted in a significant reduction of the amount of fibrinous connective tissue with a concomitant increase in loose connective tissue and the appearance of a few distinctive, compact bundles of unmyelinated nerve fibers.

Consistent behavioral modifications were not observed in any of the treatment groups which would significantly distinguish them from controls. Our results appear contradictory. Findings of Matinian and Andrianian (1976) who reported return of normal sensori-motor function in 80% of their animals treated with trypsin and cytoxan appear contradictory. The findings of Matinian and Andrianian (1976) who reported return of normal sensori-motor function in 80% of their animals treated with trypsin and cytoxan appear contradictory. Although the number of untreated control animals reviewed was not great enough to assess statistically, the severe erosion of the remaining spinal cord due to cyst enlargement was a grant from the Edward C. Schliefed Foundation.


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 a proagglutins with ensuing cellular infiltration and spread of the hemorrhagic lesion due to the following mechanism. It is known that during trauma-induced hypoxia and ischemia, Intracellular K+ is lost to the extracellular space with the loss of the membrane potential. Thereafter, extracellular potassium accumulates within the spinal cord reaching concentrations of up to 70 mM. Swelling may result from 1) uptake of K+ plus CI- in accordance with Donnan equilibrium and 2) an extracellular component of HCO3- stimulated swelling involving the uptake of Na+ and CI- into gila and possibly other cells. When swelling occurs in perivascular astroglia it can compres 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 CI- transport which can be inhibited by an unsaturated ketone derivative of arylxycetic acid (ethacrynic acid) among others. We treated the hypothesis that the CI- dependent, HCO3- stimulated component of astrogial swelling is an important factor in the evolution of spinal cord injury and that this inhibition by ethacrynic acid may improve recovery from such an injury. Anesthetized cats were injured by dropping a |5 g weight 20 cm onto an impounder and strain gauge centered on the tip of the cord. The time of the injury is recorded within 50 ms of the impulse of the blow was recorded. Animals were treated with saline or ethacrynic acid 5 min following injury. Cats were evaluated 1 hr post-injury by performing a clinical neurological and electrophysiological test. The extent of the cord injury was then assessed histologically. All three methods of assessment revealed a significant improvement in Schewitz's Motor Test. The extent of the cord injury was assessed histologically. All three methods of assessment revealed a significant improvement in Schewitz's Motor Test.
Physiological studies of single medial gastrocnemius (MG) Ia afferents were made to determine their rostro-caudal distributions, branching patterns, terminal distributions, and conduction properties of MG Ia EPSP-generating potentials in the cat spinal cord. Through the use of signal averaging techniques, field potentials from motor root fibers (PRD) and EPSPs occurring synchronously with action potentials in single MG Ia dorsal root afferents were studied in the region of the triceps surae motoneuronal pool. MG Ia afferents bifurcate upon entering the spinal cord and conduct both caudally (at 22 M/S) and rostrally (at 21 M/S). At the level of 1800 mm (X = 126 mm), 6-8 major collateral branches conduct ventrally at 8-19 mm/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).

The extent 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 8600 mm 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 Research Service 264 and VA Hospital Medical Research Service.)

**Central Terminal Excitability Changes of Single Type I Afferent Fibers.**


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 a single spike or short train of spikes, were electrically stimulated at the site of the presynaptic terminals in the spinal cord, either within or outside the grey matter, in the white matter, or in both. These results indicate that Type I afferent fibers can be driven 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.)

**Electrophysiology of Acute Spinal Dorsal Horn.**

A.C. NACHMIAU, M. BARTELS and H.-D. ROHMANN, Dept. Physiol., Univ. of Hamburg Med. and VA Hospital, Ginessville, FL 32610

An exposed, otherwise intact spinal L7 segment in the cat was subjected to a sudden-lasting depolarization by an electromagnetically driven metal rod. Pathophysiologial events at different degrees of compression were studied by recording: (a) polysynaptic reflexes and discharges (PRD); (b) central and EPSPs (CEP) in response to the same afferent volley triggering PRD; (c) conduction through the injured segment by recording for example S3 dorsal root potentials as well as dorsal column stimulation at L1. At a compression of 1 mm there was initially little change (PRD). CEPs in the medulla were diminished for a few min. At 5 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 a degree of recovery of PRD and conduc-

**Central Terminal Excitability Changes of Single Type I Afferent Fibers.**


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 a single spike or short train of spikes, were electrically stimulated at the site of the presynaptic terminals in the spinal cord, either within or outside the grey matter, in the white matter, or in both. These results indicate that Type I afferent fibers can be driven 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.)

**Dorsal Rhizotomy on the Sympathetic Population in the Saccral Secondary Visceral Gray.**


Afferent impulses from pelvic visceral structures are relayed rostrally to brainstem levels via the saccular oblong pathway. The cellular origin of this fiber tract is the secondary visceral gray located in the lateral funiculus at m 4-sacral levels immediately dorsal to the lateral 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 0.15 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 toluidine blue cross sections. At the E.M. level, a heavy concentration of dense core vesicles both in axons and in terminal boutons clearly distinguished 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 not confined to 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 the nucleus. The majority of degenerating boutons contained clear spherical vesicles and were found in association with dendrites of various sizes. These terminals were characterized by the presence of clear vesicles and were characterized by 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 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 the descending hypothalamic tract system. This work was supported in part by NIH/NS465 83754-05.

Large bilateral injections (20 μl) of a 30% solution of horseradish peroxidase (HRP) were placed in the lumbar enlargement of the spinal cord of Numbal-anesthetized rats. After a survival period of 3 hr, resection of the spinal cord, and perfusion of the entire animal with 10% buffered formalin, serial sections were cut at 75 μm from dura to the ventral surface of the spinal cord. The sections were processed with the Gomori silver impregnation for histochemical demonstration of HRP. The-labelled cells were also present in the nucleus reticularis gigantocellularis, pontis caudalis and oralis, raphe magnus, pallidus and on the hypoglossal nucleus.

To confirm that the axonal transport of HRP to the LC had proceeded in catecholamine containing neurons, 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 projection of labeled cells to the LC was marked only (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 method of Cole and Henry. 6-OHDA treatment resulted in an almost complete depletion of noradrenaline (0.292±0.010 μg/g in control animals, 0.005±0.0010 μg/g after 6-OHDA).

The effect of stimulation of LC upon the activity of spinal interneurons was investigated in urethane-anaesthetized rats. The type of neuron 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.


The 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 to form an axodendritic terminal running parallel to the axons. Reaction products showed a Golgi-like picture of the central distributions. Myelinated fiber receptive units (Type I cutaneous and G-2 hair fimbriae) 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 resection of the HRP product, thin serial sections containing the stained axons 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 were distributed in the dorsal horn. All synaptic contacts of this type of receptor were deep to the substantia gelatinosa (laminae II). All of the observed synaptic profiles contained round vesicles. The various synaptic profiles formed from a HRP-filled axon showed simple axodendritic contacts as well as complex "central" terminals which had multiple axodendritic and axosomatic synapses. Other axosomatic and simple axodendritic synaptic terminals were also observed. In laminae I and IV these terminals had synaptic profiles which were unlabeled. In laminae I, II and III, the labeled primary afferent axon was post-synaptic to other profiles. G-2 hair receptor axons in cat distribute throughout the various laminae (laminae I and IV) in a manner generally similar to that for the Type I receptor. The fiber from a cat G-2 hair fimbriae receptor also showed myriads of synaptic contacts; these were simple axodendritic and the "central" terminal with multiple axodendritic synaptic contacts have been identified. (Supported by USPHS, NINHCS research grants NS11614, NS10321, and NS11332 and a fellowship HD5526 to A.R.L.)


The time course change of the spinal cord after contusion can be dramatically demonstrated by the following model. Eighteen male female dogs were used. The 7 mm diameter spinal cord was compressed to 1 mm 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 full spinal block 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 develop after the contusion.

We have previously reported massive mobilization and accumulation of neuronal lysosomes and release of lysosomal hydrolases near the plane of the spinal cord transection which was assessed with autolysis of the spinal cord tissue bordering the cut ends of the spinal cord stumps (J.Neuroiug. 56: 197, 1977).

The time course of a controlled histochemical, 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 (AFase), used as the marker for lysosomes, was demonstrated in normal and injured spinal cord tissue. In a normal spinal cord, AFase is positive in the meninges, in the blood vessel wall, and in nerve cell bodies of the grey matter, whereas it is not in the white matter tracts. At 2 hours after contusion, large quantities of lysosomes accumulate within short segments of axons in otherwise normal-appearing white matter about 2 mm from the area of petechial hemorrhage proximal and distal to the injury. At 12 hours, the lysosome-filled axon form club-shaped axonal terminals (terminal clubs) which always point inwards towards the area of injury. At one day, such terminal clubs may reach 100 μm in diameter and begin to leak AFase into the surrounding tissue. Electron microscopic study revealed rupture of the terminal club with subsequent release of lysosomes into the extracellular space. The injured areas frequently 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 AFase originated from the circulation or from macrophages. We therefore propose that axons that degenerated in the spinal cord following spinal cord contusion originated within the nervous system. (Supported by NIH NS14413-01)


Both anatomical and electrophysiological studies have shown that descending spinal axons I A XON have systems terminate on neurons at several spinal levels via short axon collaterals arising from parent axons in white matter. Yet some cells in the lumbar sacral 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 simple axodendritic and the "central" axodendritic synaptic terminals into 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 motor neurons. These HRP-filled dendrites, as well as unlabelled white matter dendrites, have been observed in all spinal cord levels. 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. Rudomin, E. Jankowska and J. Medrãnez.

Department of Physiology and Biophysics, Centro de Investigación del IPN, Mexico I4, 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 a vestibulo-spinal (VS) fibers synapsing with motoneurons are not subjected to PD. This lack of PD could be a general feature of descending fibers. The spinal cord is therefore an interesting model to test the hypothesis that atumilation of sensory nerves increases the extra-cellular 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 excitation of both corticospinal (CS) fibers and the action potentials of spino-cerebellar fibers are markedly 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 I muscle afferents of extensor muscles terminating in that same neighboring region 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 mechanism leading to PD of RS and a aon axonal terminals within the IN. It is more consistent with the idea of a specific electrically transmitting being responsible for the PD than a functional discharging of K\(^+\) accumulation. The latent period and the excitability increase of the CS fibers in the DH may 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 (7-9 ms). It was observed that the latter was increased by a greater number of interposed interneurons. The PD of CS fiber terminals in the IN may be explained by a dual mechanism: an early depolarization resulting from the electric field interactions (or K\(^+\) accumulation) and a delayed PD probably resulting from transmitter action.

Partly supported by NIH Grant 8S 0196-07.

**1836**

**RESPONSE OF PRIMARY AFFERENTS TO CHANGES IN EXTRACELLULAR POTASSIUM CONCENTRATION IN THE BULLFROG SPINAL CORD.**

Sarah A. Shefer and Richard A. Levy.


Upon afferent stimulation, K\(^+\) accumulates specifically around primary afferent terminals. This increase in extracellular K\(^+\) concentration ([K\(^+\)]\(_{oc}\)) has been proposed as a mechanism for increasing the dorsal horn's excitability (DH, dorsal horn) to presynaptic terminal depolarization (DPR). This hypothesis assumes that increases in [K\(^+\)]\(_{oc}\) will substantially depolarize primary afferent terminals. However, measurements on other neuronal preparations show their membrane potentials to be rather insensitive to changes in [K\(^+\)]\(_{oc}\), comparable to those occurring around primary afferent terminal depolarization upon afferent stimulation. Therefore, we measured the response of primary afferent terminals to changes in [K\(^+\)]\(_{oc}\). 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 DRPs' evoked by stimulation of an adjacent dorsal root.

K\(^+\)-response curves showed that only at [K\(^+\)]\(_{oc}\) values greater than 25 mM did depolarizations approach those predicted by the Nernst equation. At [K\(^+\)]\(_{oc}\) values below 25 mM, depolarizations were smaller than those predicted by the Nernst equation, with changes in [K\(^+\)]\(_{oc}\) near rest levels causing very small depolarizations. Additional evidence that K\(^+\)-free superfuse to block any K\(^+\)-induced transmitter release, decreased the K\(^+\)-induced depolarizations. This suggests that the direct depolarizing effect of physiologically occurring [K\(^+\)]\(_{oc}\) accumulation around primary afferents may be amplified by K\(^+\)-induced transmitter release from near neurons. Even in the K\(^+\)-free condition, where both direct and indirect effects of K\(^+\) were present, we found the [K\(^+\)]\(_{oc}\) required to cause a depolarization equal to the DRP height was significantly larger than the amount of K\(^+\) found (Sytsova et al., Brain Res. 106: 413, 1976) to accumulate around frog afferent termi- nals in a single ganglion. These workers found that a frequency afferent stimulation could cause K\(^+\) accumulations of up to 9 mM.

In our preparation, a change in [K\(^+\)]\(_{oc}\) of this magnitude causes a large de- polarization of the primary afferent, similar in amplitude to the DRP. Taken together, these observations should make it possible to determine the generation of depolarization by the DRP, but may supplement PAD induced by GABA re- leased at a-xonic 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 for high-frequency afferent activity.

**1838**

**THE SPECIAL RELATIONSHIPS OF CHICKEN EPIDYMAL CELLS IN THE SPINAL CORD AND CERTAIN BRAINSTEM AREAS.**

Frances M. Samsono.


An earlier investigation has shown that glycinergic-rich cells surround epidermal cells throughout the entire length of the spinal cord and lower medulla. When the canal opens into the fourth ventricle, these cells assume a ventral midline position immedi- ately above the afferent and efferent masses of the olivocerebellar motor nucleus (Samsono, J. Morph. 153:87, 1977). It was observed in that study, that the ventricular epidermal (VE) dorsal to the glycinergic cells elevated the IPN and adjacent cells are tall columnar cells with basally placed nuclei. These nuclei form two to three layers giving the appearance of a subventricular or diffuse layer of ependymal cells that are bounded by small basilar neurons, so one is indeed struck by the similarity to the developing spinal cord. However, no mitotic figures are seen. Toluidine blue-stained sections suggest that the cells that appear show capillaries to be present just beneath the epidermal cells. At the ultrastructural level both the VE and SC cells display basically located spherical or ovoid nuclei and the apical portions are filled with an elaborate Golgi apparatus, many smooth vesicles and large numbers of lysosomes. Rare endoplasmic reticulum and gliosinfilaments are sparse. A few polymers are scattered about. Some of the glycinergic synthesize large amounts of beta glycinergic particles. Rare cilia and irregular margins characterize the luminal surfaces. Below the apical junctional complexes, their lateral borders are indistinguishable. The VE contains numerous vesicles similar to the pattern seen in mammalian tanyocytes (Millhouse, Brain-Endocrine Interaction 11:3, 1974). A Golgi-Cox impregnation of some of the epidermal cells reveals a small spherical, non-membrane-bounded vesicle oriented process which ends around blood vessels in the glycinergic area. These cells seem to fit the classification of epidermal and ciliated neuroepithelial cells, an unusual relationship between a normally ventricular ependymal cell and a normally dorsal component of the CNS. The VE and SC cells are the only non-neuronal cells in either brainstem or spinal cord. Because of the unique arrangement of chicken epidermal cells, it seems plausible to postulate that they either release a substance to the cerebral spinal fluid or transport substances from the fluid to the blood.

Neural processes containing clear synaptic vesicles have been found between the cell bodies of the VE and VE or VE and SC. The unique relationship between VE and SC and the existence of two different type synapses have been found. It is assumed that these axons arise from the contiguous small neurons. (Supported by the University of Buffalo Foundation).

**1839**

**CORRELATIVE STUDIES OF INDIVIDUAL IA-MOTONEURON PRESYNAPTIC TERMINAL SPIKES AND EPSPS.** G.W. Eptet and J.B. Munson.

Univ. of Fla. Coll. of Med. and VA Hospital, Gainesville, FL 32610.

Electrical potentials in the triceps surae motoneuron pool generally decay with action potentials in single medial gastrocnemius (MG) IA dorsal root axons recorded in cats, using low resistance impaled microelectrodes and averaging techniques. These potentials were of several types, including 1) presynaptic terminal spikes and 2) late presynaptic axonal spikes were usually a positive-negative sinusoid less than 20\(\mu\)V peak-to-peak amplitude and about 400\(\mu\)sec total duration. They were recorded from an 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. The peak latency of IA axons has been particularly interested in correlates of EPSP latency from the presynaptic terminal spike. This latency is composed of synaptic delay plus electrotonic delay and is the time from synaptic site to recording site in the motoneuron soma, and thus should relate directly to electrotonic delay. It is therefore readily recorded on an oscilloscope and is useful as an index of EPSP latency. Significant linear correlations were found between latency and amplitude of EPSP (Figure 1) and between latency and amplitude and slope of the latency line (Figure 2). The latency slope correlation was most powerful (homogeneous r, 0.65 p<0.001); heterogeneity r, .87 (p<0.0001). Thus, EPSP latency and slope of the latency line are both relevant to the possibility of electrical transmission at the IA-motoneuron synapse. (Supported by VA Hospital Medical Research Service and EV 01264).

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 area was 0.2-0.3 mm with a constant 3H concentration of 100 µCi/ml. 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 (Cowen 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 reticle (128 X 128 µm). The majority of silver grains was found over the contralateral side of the spinal cord; however, in some experiments, the number of grains ipsilaterally to the injected side was also considerably above background level. On the contralateral side, the grains were concentrated over Rassier’s laminae V, VI and VII; fewer grains were located 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-motorneurons. 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 course mainly within the contralateral corticospinal tract; some fibers of this tract apparently crossed 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.)


The rate at which substances reach a given group of cells in a multicellular organ 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 ganglia 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.

<table>
<thead>
<tr>
<th>C (51)#</th>
<th>T (63)</th>
<th>L (300)</th>
<th>S (250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/F</td>
<td>V/F</td>
<td>C/V</td>
<td></td>
</tr>
<tr>
<td>73±3  triple</td>
<td>106±4</td>
<td>95±2  51±1  66±1  104±5  73±1  31±1  64±1  29±5  74±1</td>
<td></td>
</tr>
<tr>
<td>30±1</td>
<td>22±1</td>
<td>26±1</td>
<td>29±0.5</td>
</tr>
<tr>
<td>2.5±0.1</td>
<td>4.7±0.3</td>
<td>3.0±1</td>
<td>1.8±0.4</td>
</tr>
</tbody>
</table>

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 mean ± SE.

# 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 BMC circulation.

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 U69; a thionoxypentobarbitone sodium (1-2 mg/kg) ip given co-transcutaneously at L-1/L-7/13 and preparation made for extracellular recording of Renshaw cell bursts evoked by supramaximal antidromic stimulation of isolated ventral roots (S1-S5) in anaesthesia.

On-line computer analyses of Renshaw cell discharges (1st and Oth order PST histograms) were made. Stimulation rates varied from 20Hz to 50Hz 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 70Hz). 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 discharge 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 almost a delay of 20-30 seconds and responses to individual stimuli (20 Hz) began after delays of 50 msec. or greater, again directly dependent on the duration and frequency of stimulation.

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 U69; a thionoxypentobarbitone sodium (1-2 mg/kg) ip given co-transcutaneously at L-1/L-7/13 and preparation made for extracellular recording of Renshaw cell bursts evoked by supramaximal antidromic stimulation of isolated ventral roots (S1-S5) in anaesthesia.

On-line computer analyses of Renshaw cell discharges (1st and Oth order PST histograms) were made. Stimulation rates varied from 20Hz to 50Hz 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 70Hz). 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 discharge 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 almost a delay of 20-30 seconds and responses to individual stimuli (20Hz) began after delays of 50 msec. or greater, again directly dependent on the duration and frequency of stimulation.

ULTRASTRUCTURAL CHANGES CAUSED BY SPINAL CORD ISCHEMIA, Shokei Yamada*, Robert L. Schultz*, (SPBH: 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,a2 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 cadocylate-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 cristae of the endoplasmic reticulum were obliterated in some areas, and the membranes close together. However, 3 and 5 minute ischemic cords did not show a 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,a2, and in interneuronal activity of the spinal cord produced by 15 minute occlusion of the descending aorta.

Online, real-time computer monitoring of single unit activity in the mammalian spinal cord. Robert P. Tejierski, Richard T. Gumble* 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 PORTRAY 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 dorsalventral 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.

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 (DSC) 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 percent 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
Ammonium (NH₄) acetate and chloride have been shown to reduce or eliminate hyperpolarizing IPSPs in several systems, including spinal motoneurons (NH₄) (Lux 1972), trochoarachnids (Linna, et al. 1974), neocortical cells (Rasche and Guthit, 1975) and crayfish stretch receptor and crustacean heart (Lux 1974). However, doses of NH₄⁺ effective on these neurons (i.e., 1-4mM Kg) have little effect on hippocampal IPSPs in vivo (Eccles, et al. 1977). We have examined the action of NH₄ 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 impairing a CAI neuron with a potassium methysulphate filled electrode, NH₄ was applied by perfusion at concentrations from 4-8mM. Epss was determined by applying hyperpolarizing current pulses through the electrode. Because the presence of EPSPs can complicate measurements of Epss, pentobarbital (10⁻⁴mM) was used to prolong the IPSP and permit IPSP measurements to be made at times when IPSPs would be minimal. Pentobarbital also reduces the seizure activity occasionally seen in NH₄. All doses of NH₄⁺ had a generalized depressive action on excitability in pentobarbital, resulting in abolition of the field potential population spike within 8 min at 8mM. Perfusion with 4mM NH₄⁺ for at least 10 min had little effect on Epss, while perfusion for 20 min caused a slight and variable reduction (ca.10-20%). NH₄⁺ caused a rapid (c.Smin) and reversible depolarization (ca. 5mV) and a reduction of about 50% in Epss. A similar depression of double shock inhibition of field potentials by NH₄⁺ has the same effects as an IPSP recorded intracellularly, was not seen with 8mM NH₄ at times when NH₄ had reduced the original population spike amplitude by 75%. In conclusion these results indicate that doses of NH₄⁺ 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 Epss in this system. The failure of NH₄⁺ 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.

We find that restricted sets of synaptic terminals made by the excitatory axon on crayfish (Procambarus clarkii) opener muscles of the chelifed show very different firing characteristics for different stimulus rates and for different stimulus patterns at a given rate. Furthermore, the amount of facilitation at a given set of synapses may vary according to which facilitation is measured. For example, very different values for facilitation may be obtained if one measures the amplitude of the nth pulse, the difference between the amplitude of the first pulse in a train; or 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 excitatory axon via its pre-synaptic effect on the excitatory axon. However, if the inhibitor and excitatory axons are stimulated together at an optimum timing for pre-synaptic inhibition and the inhibitor stimulation is suddenly stopped, the excitatory axon at the next pulse in the train often releases the same amount of transmitter it would have released if only the excitatory axon had previously been stimulated. This result implies that facilitation processes in which more depolarization of the presynaptic terminal nerve, the amplitude of the pre-synaptic stimulation. This result is in agreement with facilitation at squid synapses (Chariton and Bitner, J. Gen. Physiol., in press).

**BIOCHEMICAL CHARACTERIZATION OF CYTOCHEMICALLY LOCALIZED CYCLIC NUCLEOTIDE PHOSPHODIESTERASE.** Harlton A. Armano and M. Michael Appel. In this study, a series of biochemical characterization of the cyclic nucleotide phosphodiesterase (PDE) activity has been cytochemically visualized at postsynaptic sites of asymmetrical terminals within the neuropil of the rabbit corpus atrium of the cerebral hemisphere and crustacean heart. A series of an electron-dense PDE reaction product occurs following incubation of 100 um tissue slices of aldehydes-fixed brain with cyclic 3',5'-guanine monophosphate (cyclic GMP) or cyclic 3',5'-adenosine monophosphate (cyclic AMP) as substrates. Visualization of the cyclic AMP 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 cerebellum, prepared by homogenization, sonication, and centrifugation shows the presence of three discrete activity fractions. This enzyme is strongly inhibited by the phenothiazine, trifluoperazine, but the inhibition can be overcome by increasing the amount of protein activator. The second active form of DEAE fraction is a cyclic GMP-specific phosphodiesterase, requiring calcium and a protein activator for full activity. This enzyme is non-synaptically inhibited by the phenothiazine trifluoperazine, but can be significantly activated by cAMP and a protein activator or significantly inhibited by trifluperazine.

**KINETIC ANALYSIS OF TISSUES PROCESSED FOR ELECTRON CYTOCHEMISTRY.** Cyclic nucleotide phosphodiesterase activity at synapses terminals is modulated by the concentration of cyclic nucleotides is 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.

**DEPOLARIZATION-RELEASE COUPLING AT A SYNAPSE LACKING REGENERATIVE SPIKES.** A. R. Bight and E. Linding. Dept. Physiology and Biocenics, N.Y. Univ. Med. Ctr., 550 First Ave., New York 10016. 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 polarized in the stretch reflex. A parallel ultrastructural study demonstrated that the sensory fiber is a cyclic GMP-synaptic junction with the motoneuron dendrites within the thoracopod ganglion, the presynaptic region being a simple cylinder 40-60um in diameter and 0.200um in length. The large synaptic terminal 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 of synaptic junctions within the single cell artifactic and postsynaptic recording and synaptic 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 TX poisoning of the voltage-dependent sodium conductance) follows the same bell-shaped curve, with quantitatively similar levels to those in the squid, peak-release, and suppression-potential (Lindsay et al., Proc. Natl. Acad. Sci. USA 73: 2916-2922, 1976). Also, in agreement with these findings, a maintained, presynaptical release was found to give a linearly rising post-response. Peripheric conduction in the sensory fiber has been thought to occur with simple decremental conduction but the graded synaptic-dependent voltage transient in the fiber to step-depolarization may serve to compensate for the capacitative distortion of the signal which arrives at the synapse, and at the same time very fast voltagedependent inward currents that are known in the synaptic terminal was revealed by the intracellular injection of tetrathylenonum which allowed the development of regenerative calcium spikes with large continuous depolarizing events.
1849 MODIFICATION OF SYNAPTIC TRANSMISSION AND BURSTING PATTERNS IN APYSILA 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 post-synaptic symptoms of neuronal 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 produced by altered ionic permeability in the membrane 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 Apysila californica when the pleurovisceral connective is stimulated.

The cell was hyperpolarized to -100 mV by passing current through one of two intracellular microelectrodes to suppress 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 (f2 = EPSP/EPSP1) and the 100th to that of the first (f100 = EPSP/EPSP100). These experiments were repeated at 10 ATA of air pressure, in 2% oxygen nitrogen gas mixture, and in 1/3 Ca++ solution. Application of pressure had no effect on the initial facilitation f2. However, the facilitation of the 100th EPSP, f100, was increased by 17 ± 5% (S.E.). Similar results were obtained with pressure in 1/3 Ca++ solutions and with 2% oxygen nitrogen as the pressure medium. The 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 ± 7% (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 NO0014-75-C-0547 by NIH grant NS 11255 and NIH award NS 70631 to JEB.

1850 INCREASE IN CYCLIC AMP IN RAT HYPOTHALAMUS FOLLOWING THE ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN. H. C. Buszman* and R. A. Browning (SPON: R. P. Lehr, Jr.). Southern Illinois University School of Medicine, Carbondale, IL 62901.

In recent years considerable evidence has accumulated implicating the cyclic AMP as a post-synaptic mediator of several neurotransmitters including norepinephrine and dopamine. In the case of serotonin, however, the evidence is far less conclusive. Most studies have shown that injection of cyclic AMP into the hypothalamus produces in response to serotonin, although a recent study demonstrated the presence of serotonin sensitive adenylylcylase activity in rat hypothalamus (Schoultz et al., Life Sci., 19, 191, 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. Truant et al. (J. Exp. Ther. 11, 28, 1976) demonstrated a marked supersensitivity of central serotonin receptors in response to low doses of 5-hydroxytryptophan (5-HTP) following destruction of hypothalamic serotoninergic neurons by 5,7-dihydroxytryptamine (5,7-DHT).

Two hours following pretreatment with the catecholamine uptake inhibitors, protargoline (20mg/kg, i.p.), male Sprague-Dawley rats (190-200g) were treated intravenicularly with 5,7-DHT (150µg) or vehicle. Ten to twelve days later animals were given an intraperitoneal injection of either 5-HTP (100µg/kg) or saline and sacrificed by focused microwave irradiation to the head. Endogenous cyclic AMP levels were measured by a modification of the 125I-radioimmunoassay of Steiner et al. (J. Biol. Chem., 249, 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 hypothalamus in rats receiving a saline or 5,7-DHT injection. Moreover, rats not treated with 5,7-DHT failed to exhibit an increase in cyclic AMP in either hypothalamic or caudate nuclei areas. These findings indicate 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 HALOTANE ON SYNAPTIC TRANSMISSION IN A NAMIBIAN SNAKE, BOTHRIOCEROS CAUDATUS. Deryl C. Schoultz, Dept. Pharmacology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The activity 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 post-synaptic potentials (e.p.s.p.s.) with no significant change 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 of time-course of the action potential which was evoked by direct stimulation through the recording electrode. Also, there were no changes in the hyperpolarizing electron potential, indicating that halothane had no effect on the time-constant or effective membrane resistance of the preganglionic 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 and duration of the depolarization effect of acetylcholine on the membrane. These results indicate that the effects of halothane on the superior cervical ganglion are due to alterations of the processes of synaptic transmission, and that these processes are altered by the depression of the post-synaptic 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. Frederick* and Wendy B. Yohn*, 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 and increased the membrane amplitudes of specific vesicle proteins, especially proteins DPM-1 and DPM-2 (Neurol. 28: 367, 1978; BBRC 80: 183, 1978; Epilepsia 18: 357, 1977). These results suggest that this effect on the vesicle-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 release phosphorylation without significantly affecting the norepinephrine concentration of the treated vesicles. When the solubilized vesicle fractions was added back to the calcium-homogenate, the ability of calcium to stimulate release and phosphorylation was restored. A highly purified activator protein was isolated from the solubilized fractions of the calcium-homogenate that restored the ability of calcium to stimulate neurotransmitter release and protein phosphorylation when added to the treated vesicle preparations. One effective activator protein was dependent upon the presence of physiologic intracellular concentrations of magnesium ions in the isolation media, accounting for the variable degree of calcium-sensitive 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 extend and support 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 chemical 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 I. Bailes*, 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 membrane resistance (30 uM Mg) and are blocked by exposure to muscimol 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 occurring 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 (sodgrass et al, this meeting) indicate that GABA is the predominant inhibitory transmitter utilized by mammalian cortical neurons in vitro.


Achetylcholine (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 absence of chromanol (5 uM), ACh blocked the depolarizing effects of nerve stimulation. ACh induced a hyperpolarization of the neuron membrane. ACh was administered to the solutions already present in its initial hyperpolarization followed by a depolarization. Atropine (1 uM) blocked both the depolarizing and hyperpolarizing response to ACh. On the other hand, a low Ca ++ solution, tetrotoloxin (0.1 uM), haloperidol (0.1 uM) or phenoxybenzamine (1 uM) selectively and reversibly abolished ACh-induced hyperpolarization, not affecting the depolarization. The membrane resistance remained relatively constant during the course of hyperpolarization. Application of steady depolarizing and hyperpolarizing currents 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 cholinergic adenominergic interneuron interposed between the preganglionic fibers and principal ganglion cells: when activated mechanically 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.


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 S-phenylisopropyl adenosine was considerably more potent than the corresponding D-form. Suppression of methylxanthines, which block adenosine receptors, or adenosine deaminase, which catalyzes extracellular adenosine, profoundly antag- onizes depressant adenosine responses. Both methylxanthines and adenosine deaminase augment the amplitude of the evoked field potentials when administered alone. Hexobenzine, 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-cyclic 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 Schaffer-commissural system, and that endogenous release of adenosine or adenosine 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 activity 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 T01 GM01983.

1856 RESPECTIVE ROLES OF ELECTRICAL AND CHEMICAL FEEDFORWARD INHIBI- TIONS IN REGULATING RESPONSES OF THE GOLDFISH MANTHUR CELL TO EXCITATORY AFFERENTS. Donald S. Faber and Henri Korn. N.Y. Res. Inst. on Alcoholism and Dept. of Physiology, SUNY, Buffalo, N.Y. and INSERM U. 13, CHU Pitié-Salpêtrière, Paris, France.

Field effect and chemical inhibitions of the goldfish Mauthner cell (M-cell) are mediated by impulses in the same interneurons (hippocampus) of its recurrent collateral network (Science, 1976, 194: 1160). The hypothesis that these interneurons also exert a feedforward inhibition of the M-cell has been confirmed by comparing their intracellulack recorded responses to stimulations of the ipsilateral posterior eighth nerve, which has well-established electrotetrotonic 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).

Eight nerve stimuli evoked both electrical and chemical inhibitions of the M-cell: 1) the electrical component is a short latency (0.25 msec) excitatory depolarizing potential (EDP) recorded extracellularly in the M-cell axon cap. Its threshold was 0.2-0.25T and it was maximal in amplitude (6-8 mV) with stimulus strengths of 1.0T or less. 2) 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 mono- synaptically mediated (PSPS). Threshold intensity for the excitatory responses was also comparable to that of the inhibitory ones, but with greater variability in amplitude and latency. Since EDP peak time (0.65 msec) is intermediate between those measured for the electrotonic and chemical excitatory components (0.35 and 1.0T), respectively, the field effect on the M- cell excitation at intensities below 1.0T may even produce a transient net membrane hyperpolarization. During the same experiments 90% of the M-cell potentials recorded in the presence of a much lower stimulus (0.3-0.6T) than did the M-cell, and the latency of their activation corresponded to that of the EDP, a finding consistent with the hypothesis that their orthodromic impulses mediate at least a portion of the afferent inhibitions. Finally, simultaneous intracellular recordings from the M-cell and these synaptic 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 inhibition would not be expected to 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).

The opener muscle of the crayfish claw is innervated by only two axons, an excitatory and an inhibitory. The inhibitor axon innervates not only the opener muscle fiber, but the terminals of the excitatory axon as well; thus providing both post- and presynaptic inhibition. Intracellular recordings from the excitatory 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 openner nerve near the intracellular electrode. Using this technique, it is possible to obtain a reversal potential for the IPSP in the excitatory 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 extracellular 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 excitatory axon. 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, Pflugers Arch. 364: 217-222, 1976; Marder and Paupardin-Tritsch, J. Physiol in press, 1970.)


We have studied stimulus-secretion coupling in the salivary gland of the slug, Arionia californica. Histological examination of the gland has demonstrated at least four secretory cell types based on cell shape 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 or electrophysiological techniques to be applied simultaneously. Cells in the intact gland have been studied with standard electrophysiological techniques.

Many cells have resting potentials of 60-80 mV and generate all or none, overshooting 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 Ca 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 show physiological changes which may be related to the secretory process.

1860 GLUTAMATE AND SYNAPTIC DEPOLARIZATION OF CEREellar PURINeJE CELLS. A. Godfraind, J. Guitton and F. Giraudon. 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 potential (PSP) in PC's, which can be reversed with extrinsic polarization through the iontophoresis of a charged chloride ionomer at intermedial at three synapses. The PC-PSPs evoked by electrical stimulation of PP's usually have a more negative reversal potential than that of PP's elicited by CF activation. This difference in reversal potentials provides a basis for characterizing agonists of the PC synaptic receptors. Accordingly, we have investigated the effects of Glu on 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 μM GABA. The GABA selectively increased PC ionic conductance that blocked PF-PC transmission with little or no effect upon intracellular recorded CF-PFP's. Three types of synaptic contacts upon the PC were seen at the electrophysiological level: 1) PF's contacted spines on the spiny branchlet units of the distal dentrites; 2) the CF contacted thorns emitted from the soma and proximal dentrites; 3) the SC contacted either the PC axon hillock, soma, and dentrites. The geometric distribution of these contacts suggests that GABA may act upon the PC dendrite where these SC contacts occur. This increased conductance would tend to shunt distal excitation, and thus restrict PF synaptic current to the molecular layer, while the CF evoked excitatory postsynaptic potentials near the PC soma. The PC-PSPs are depolarizing elicited by Glu and PF stimulation had similar reversal potentials while CF-PSP's were elicited at a lower membrane potential, though the values were not significantly different. The negative value of the reversal potential indicates that there are both sodium and potassium ionic conductance channels opened by the action of PF's. The results are consistent with the hypothesis that Glu and PF transmitter act on the same PC synaptic receptor, suggesting in fact that Glu may be a PF neurotransmitter.

Supported by RDA 51052 DA 00009 from NIMH 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. Jeffe, Richard D. Phillips* and William T. Kaune, Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratories, Richland, WA 99352.

The effects of chronic exposure to a 60 Hz electric field on synaptic transmission and peripheral nerve function in the rat have been studied. The results of these experiments suggest that chronic exposure to a 60 Hz electric field may affect synaptic transmission and peripheral nerve function in the rat.

1862 RELIABILITY OF ACH FROM GANGLIONIC "SUBCUTANEOUS" CELL SOMAS. David A. Johnson* and Guillermo Pilar (SPIM: C: U; 81:001). Physiology Section, Biological and Medical Sciences, University of Arizona, Tucson, AZ 85721.

Acetylcholine (ACh) release from neuronal cell somas was examined using the chick ciliary ganglia. Cell bodies in this ganglion are known to synthesize and store cholinergic transmitters. Stimulation of the ganglia in 3-5 mM acetylcholine, which was preincubated in a medium containing 10 mM potassium and 0.2 mM calcium, produced a wave of depolarization that was followed by a burst of action potentials. The results suggest that the release of ACh from somatic neurons is mediated by excitatory cholinergic neurotransmission.

1863 POLYPEPTIDE NEUROTOXINS FROM SCORPION AND SEA ANEMONE ACTIVATE NEURONAL SODIUM CHANNELS BY SIMILAR MECHANISMS. Bruce K. Kroger and Nordeci P. Blautstein, Dept. Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Polypeptide toxins from the scorpion Scolopendra hermaphrodita (LTq) and the sea anemone Anthopleura xanthogrammica (AATx) have been shown to activate neuronal sodium channels by similar mechanisms. The toxins induce a rapid and reversible increase in intracellular calcium and an increase in membrane excitability. These results suggest that the toxins may play a role in the release of neurotransmitters.

1864 LOCAL SYNAPTIC CIRCUITS IN RAT HICCUPPOCAL SLICES: INTERACTIONS BETWEEN PYRAMIDAL CELLS. B.A. MacVicar and K.K. Dukas, 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 and recording from single cells. Synaptic physiology of intrinsic hippocampal pathways was studied in vitro with simultaneous intracellular recordings. Conclusions drawn from this studies indicate that excitatory postsynaptic potentials (EPSPS) are mediated by glutamate as the neurotransmitter. These findings support the idea that the hippocampus is involved in the generation of the hiccups.

---

*Supported by EPSRS grant NS-08442 and fellowship NS-05112.

In the last twenty-five years, studies of biochemical and pharmacological correlates of affective disorders have yielded and expressed impressive data. However, the mechanisms and pathogenesis of affective disorders unknown, but the mechanisms by which pharmacological agents are able to ameliorate the symptoms remain obscure. Lithium salt has been successfully employed in the treatment of bipolar disorder, 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 (e.g., calcium), and involvement with transmitter metabolism (serotonin). Intracellular and extra cellular recording is in progress in the invivo hippocampal slice preparation and provides evidence for the relative importance of several component mechanisms in the synaptic response. The 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 in and mode of action is known, may provide an effective technique in dissecting the synapse.


The effects of -aminobutyric acid (GABA), glycine, and (4-Chlorophenyl)-y-amino butyric acid (Baclofen) were observed on (i) the excitability of primary afferent fibres, and (ii) the transmission through central synapses of the deafferent cat. In 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 (Krinjevic & Morris 1976, J. Physiol. 275: 791; Morris 1978, J. Physiol. 280: 83).

Glycine (0.1-3.2 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 (0.1-2.0 g/kg i.v) or 10^7 M superfusion of the medulla caused a large, reversible increase in afferent and peripheral fibre excitability; synaptic efficiency either fell or was enhanced. During the GABA-evoked changes corresponding increases in both the extracellular calcium level to 1.5 mmol/L were measured in the cuneate with ion-selective micro-electrodes. GABA's direct action on peripheral fibres was confirmed in experiments with the isolated frog sartorius nerve, by observing changes in compund action potentials, which were evoked by Hz stimulus intensities which were submaximal for A fibres. During the calcium increase in extracellular nerve, increased by applications of 10^7-10^8 M GABA to the site of stimulation, they could be blocked by picrotoxin. The depression of cuneate transmission which glyceine produced was entirely post-synaptic. A component of Baclofen's main presynaptic action (Fox et al. 1976, Neuroscience IN PRESS) may be a depression in afferent activity reaching the Ong. 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 may be instrumental in extra- and post-synaptic effects. The degree of Blockade of the AP conduction, the effects on the neurotransmitter release, and the effects on the postsynaptic membrane, may be partially determined by the extracellular K⁺ accumulation, arising either from a direct membrane action and glial uptake of K⁺.


At crustacean neuromuscular junction, the excitatory response of the muscle to glutamate is markedly potentiated by aspartate which has only a small effect when given alone. We have investigated the modulation of glutamate responses by aspartate on Aphylla neurons. However, these glutamate responses are due to either CI⁻ or K⁺ conductance increases rather than a Na⁺ conductance increase in the crustacean synapse. Many of our recordings were made from unidentified neurons in the buccal ganglion and some from the abdominal ganglion. Cells were penetrated with two independently immobilized microelectrodes for simultaneous monitoring of glutamate responses to CI⁻ and K⁺. The Na⁺ responses are rare and we have not investigated those in detail. For most CI⁻ and K⁺ responses, aspartate is a 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 pre-conditioning 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 CI⁻ 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 CI⁻ and K⁺ responses may actually increase in size under these circumstances. In addition, causing an increase in seawater response the 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 depolarizing modulation in most the modulation is still present at temperatures as low as 5°C. Cystine-sulfonic acid analogue of aspartate, also causes modulation of the glutamate response and is about equally effective as aspartate. It is of interest that homocysteic acid, a sulfonic acid homologue to glutamate, blocks the glutamate responses and inhibits the facilitation by aspartate.

Some authors have observed the desensitization of the glutamate response by aspartate to a conformational change, 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.


The effects of the putative calcium channel blockers, verapamil (VPL) and D-600, were tested on pinched off presynaptic nerve terminals from rat brain gdp on the frog sartorius nervomuscular junction (n.m.j.). Ca uptake (J. Physiol. 241: 617); Verapamil (VPL) was depolarized either with verapamil, an alkaloid that opens sodium channels, or with high external potassium concentrations. The extent of synaptic depression was determined indirectly with the voltage-sensitive fluorescent dye, di-pentyl acarbocyanine (J. Physiol. 241: 589, 1972). VPL or D-600 (40-60 μM) inhibited the potassium-induced 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 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 miniature end-plate potentials (mep's) were used to evaluate the actions of VPL and D-600 at the frog n.m.j.. Low concentrations (5-20 μM) modestly potentiated the effect on mep amplitude, and either slightly decreased (<25%) or had no effect on the mep amplitude. These concentrations of drugs did, however, have a marked inhibitory effect on Ca accumulation at 20°C. This effect was abolished by 4°C, suggesting that no effect on n.m.j. at 5°C. These findings suggest that the VPL and D-600 can block sodium channels in the nerve. In preliminary where mep frequency had been depressed by the drugs, the frequency returned to normal following Ca calcium concentration by raising the external potassium, VPL (40-50 μM) and D-600 (10 μM) did not depress mep frequency. Although VPL and D-600 were able to alter the threshold for inward calcium currents in mammalian myocardium (Ehohhardt et al., Pfluegers Arch. 335: 309, 1972) our data indicate that these drugs block the threshold for Ca 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 NSF grants NSB-86424.)
1969 AMINE MODULATION OF RATE OF DECAY OF POST-TETANIC POTENTIATION IS MEDIATED BY ACYCLIC NUCLEOTIDE. S. A. Newlin, W. T. Schiaffler, and S. E. Barondes. S. J. Physiol., 29, 111 (1969). We have previously presented evidence for serotoninergic and dopaminergic heterosynaptic modulation of the rate of decay of post-tetanic potentiation (PTP) at the calyceal synapse (RCl-R15) in the abdominal ganglion of Aplysia californica (i.e., amines speed the rate at which the potentiated epp falls to its resting value). Following repeated stimulation, the presence of serotonin antagonists (SHT) and chronic exposure to isobutylmethylxanthine (IBMX; 5x10^{-4} M) and RO 20-1724 (10^{-4} M) depress the effects on PTP decay of perfused amines or neuronal activation. This effect is distinguishable from the presynaptic effects on PTP decay rate). Effects of the amines as well as the analogs 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 represents 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 VAM, San Diego, and a grant from the NIAAA.

1970 COLCHICIN-LIKE EFFECTS OF 8-B-BUNGAROTOXIN. Ronald H. Ng, Keith Terasaki* and Bruce D. Howard. Dept. of Biological Chemistry, UCLA, Los Angeles, Calif. 90024. 8-Bungarotoxin (M.W. 26,000) is a presynaptically acting, neurotoxic, protein from snake venom. It causes neuromuscular blockade and ejection of nontransmitter ACh. Collis and y are protein toxins made by Esherichia coli. These collis are known to interfere with several bacterial transport processes and decrease ACh release. The effects of 8-Bungarotoxin on synaptosomes in a manner similar to that of amines, but also to use 8-Bungarotoxin in combination with the ATPase(s) of synaptosomes. Our studies indicate that the 8-Bungarotoxin-induced interference with synaptosomal transport processes and reductions 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 10197 and NIH postdoctoral fellowship 50706.)


1971 ELECTROPHYSIOLOGICAL STUDIES IN THE ISOLATED TURTLE BRAIN. H. C. Nuschkys, H. Wachtel and M. N. Shephard. 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 allow electrophysiological analysis of neuronal properties and under more controlled conditions than are found in vivo. We report here preliminary results from studies on isolated brain of the turtle, Pseudemys scripta. The brains 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 partial pressure of oxygen in the brain of interest was 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 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. 239:60-61F, 1974), and the in vivo rabbit olfactory bulb (Getchell, S. Physiol. and Pharmacol., 29:85-98, 1975). The synaptic-evoked potentials typically undergone long-lasting suppression, for periods of up to several seconds. Extracellular single unit recordings show that the suppression is due to dendro-dendritic synaptic interactions between mitral cells and interneurons. The present results suggest that these interneuronal synapses are effective in the initial preparation. This is an important criterion for the viability of the preparation, and its validity for the analysis of local circuit properties.

1972 EXTREME SENSITIVITY OF Olfactory CORTICAL NEURONS TO KAINIC ACID TOXICITY. J. M. Korf, T. de Groh 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 diencéphalon. 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, intratraentricular 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 moles in 0.5-1 μl) unilaterally into the diencéphalon about 3 mm from PVH but somewhat 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 intratraentricular injection of KA (1.5-10 moles) revealed extensive OC damage. We have been using the KA (2-5 mg) and smaller doses of the altered intraven- tricularly sometimes cause OC lesions. The observation that KA induces degeneration of OC neurons following various modes of administration, including systemic and direct injection into brain regions quite distant from OC, implies only the 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 importance of neurotoxic 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 glutamatergically 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 Grant NS 09156, IA-00259, RI-07066 and RSD Award M9-58884 (J.M.O.)

Hsiao and Pranger have described a toxic protein, leptotininarin (LPT), which occurs in the hemolymph of Leptotinaria halmo- ni (Hsiao, In: Tissues Animal, Plenum: F. Rosenberg, Perignon Press, 1978). We have improved the original fly lethality assay for the toxin, and have studied many of the properties of LPT in normal nerve-diaphragm preparation from the rat. Intracellular recordings with conventional 3 meq- ohm microelectrodes filled with KC1 show that treatment with partially purified LPT (0-200 fraction; Hsiao and Pranger, Toxi- con 7, 119, 1969) produces a massive outpouring of miniature end plate potentials (mepps) reminiscent of the action of black widow venom gland homologues (BWGH). Unlike BWGH, LPT induces a bi- phasic release: about 5% of the total size 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 5800 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. No further different pools of presynaptic ACh can be released in a quan- tized manner, or (b) release from a single pool can be stimulated by two different mechanisms, both of which yield quantized re- lease. Studies with the purified toxin should be of value in our understanding of the control release of ACh. Supported in part by the National Science Foundation (BNS 76-0657) and the Wilson Research and Development Co.

1874 DISTRIBUTION AND PHYSIOLOGICAL PROPERTIES OF IVN THROUGH-FIBER STRAPSES IN THE STOMATOGASTRIC GANGLION. Karen A. Sigvardt and Brian Mulloney. Dept. of Zoology, University of California at 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 partic- ular distribution of activity of the IVN fibers innervating each 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 predom- inates at low frequencies but is less effective at frequencies above 25 Hz. At higher frequencies the slower inhibitory compo- nent dominates. The IPSP involves a mixed Cl- and K+ conduc- tance increase. Its reversal potential is -74 mV and is changed in the depolarizing direction by increasing external K+ concen- tration 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.


Biological characteristics of GABA neuronal function were studied in low density dissociated rat cortical cell cultures (Dichter, Brain Res., 1979). 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 HO-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 3H-glycine. We believe that, in our cultures, glycine acts as a co-transmitter. Tyrosine hydroxylase activity is not detectable in the cultures, in keeping with the presumed absence of monoamine cells or 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 STRAPSES. J. B. Susakova*, (spon. T. L. Schwartz) Physiology Section, Biological Sciences Group, Univ. of Conn., Storrs, CT 06268.

Recovery of synaptic vesicles in the electromotor nerve terminals of Torpedo was studied on the ultrastructural and bio- chemical 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 3 hours. Over the same time period, the ultra- structural 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) Re- formation 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 en- trance of the dextran in the reformed vesicles could be demon- strated. This and other morphological evidence suggest that after intensified vesicle usage, new vesicles may originate from cytoplasmic mem- brane of neuron structures rather than by di- rect retrieval from plasmalemma. The present results, when com- pared with results obtained by Zimmerman and Denetong (Neuro- science 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 NS01368.
EVIDENCE FOR THE LACK OF INVOLVEMENT OF CYCLIC AMP IN MODULATION OF GANGLIONIC TRANSMISSION IN THE GUINEA PIG SUPERIOR CERVICAL GANGLION

James K. Wamsley*, Ann 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, norepinephrine, or isoproterenol on ganglionic transmission were tested. Medium containing 5 μM 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 ρ-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 ρ cell, dopamine receptor—adenylate cyclase complex, and the generation of a slow inhibitory post-synaptic potential (s-EPSP). Previous research has indicated that the CAMP theory of neuromuscular transmission is not confirmed by the present data. Therefore, that the SCG is not involved in the mediation of neural transmission in the guinea pig SCG (like the rabbit SCG, where it has been shown to have this function).

Supported in part by National Research Service Award PHS 17-326L6317-2, R.K.W., a Pharmaceutical Manufacturer's Association Foundation, Inc., Research Starter Grant to A.C.B., and by NS-11650 to T.W.H. from the National Institutes of Health.


Disassociated embryonic rat superior cervical ganglion neurons (scg) form cholinergic synaptic connections on each other (O'Lague et al., PNAS 71: 3602, '76; Ro et al. Br. Res., 117: 447, '76), as well as on muscle fibers from adult rat diaphragm (O'Lague et al., PNAS 72: 1955, '75). Hexamethonium (C-6), a ganglionic blocking agent, antagonizes the former synaptic potentials more effectively than it does the latter (Viets, et al.) (Viets, et al., '75). Evidence from our laboratory has indicated that normally adrenergic scg acquire the ability to form cholinergic synapses in the absence of the presynaptic Ca++ in vitro (Johnson et al., Nature, 262: 308, '76). Recent work on explants of scg taken from progressively older postnatal rats has shown that the acquisition of a presynaptic ability to form cholinergic synapses in vitro (Johnson et al., Nature, 262: 308, '76).

Since 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 scg even when taken from 12 week old rats. The epsps were blocked by C-6; a few potentials could be blocked by the β-adrenergic antagonist propranolol. In some cultures, many as 80% of the neurons penetrated were synaptically coupled to other interneurons. Since 5-10% of older rats elicited d-tuc (1 μM) sensitive functional synapses with skeletal muscle; these were often suprathreshold. Several scg formed monosynaptic junctions and an autapse. Autaptic potentials were not effectively blocked by 1 μM d-tuc. The data suggest that either the same subpopulation of cholinergic sympathetic neurons selectively survive in these cultures, or that, in contrast to the sciatic nerve of the newt, there is a specific degree of receptor transcription into adulthood. Studies are in progress to differentiate these two possibilities. (Supported by NIH grants 11888 and NS 09809)


Muscarinic cholinergic receptors regulate cyclic AMP levels in bovine SCG, where muscarinic agonists increase cyclic AMP levels of principal ganglionic neurons (PGNs) (1,2). Current evidence suggests that acetylcholine released in ganglionic terminals interacts with muscarinic receptors, causing increased cyclic AMP levels in PGNs, resulting in generation of a slow excitatory post-synaptic potential (s-EPSP). This would be a role for 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 a series of cyclic AMP agents on excitation of this ganglion cell system in vitro. Guineapig SCG were pre-incubated in Eagle's Medium (EM) for 20 min. at 37 C., then transferred to EM containing 5 μM theophylline, 2.2 mM Ca++ and 2.2 mM Mg++. SCG were incubated for 2 min. and frozen in liquid N2. Preganglionic physiological stimulation was performed at 37 C. in EM containing 5 μM theophylline and 2.2 mM Ca++. STIMULATION PROTOCOL: 2 min. 500 μM Carbachol 2.97 ± 0.5 (8)* 2 min. 500 μM Carbachol + 300 μM Atropine 0.60 ± 0.05 (11) 8 min. Incubation Control 0.47 ± 0.14 (8)* 8 min. Preganglionic Stimulation (10 Hz) 1.40 ± 0.17 (10)* 8 min. Incubation Control 0.50 ± 0.09 (9) Atropine 0.60 ± 0.05 (11)

*P<0.05 Significant Difference (Student's t-Test).


Supported in part by National Research Service Award PHS 17-326L6317-2, R.K.W., a Pharmaceutical Manufacturer's Association Foundation, Inc., Research Starter Grant to A.C.B., and by NS-11650 to T.W.H. from the N.I.H.

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. Micromanipulation 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 electrophysiologically 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 somal 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 AI 16813 and Research Career Development Award AI 70726 to J.D.W.

1882 LEPTINOTARSA: 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 neurotransmitters which influence the spontaneous release of neurotransmitter, such as black widow spider toxin and B hahemani, 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, leptomotarasin (LPT). LPT is a protein which was discovered by Hsiao and Frankei (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. hahemani to stimulate release of acetylcholine (ACh) from rat brain synaptosomal preparations. Synaptosomes were incubated with [3H] 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 SD7 RB07012), and the Nelson Research and Development Co.
TISSUE CULTURE

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 neurite outgrowth, 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 populations or (2) a combined culture containing both cell types. A combination of substrates of neurite outgrowth (glial-fibronectin) with a horse-serum containing 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 populations of NR cells can also be obtained (Supported by USPHS grant NS-07606).

LABELING CHOLINERGIC NEURONS IN CELL CULTURE. Kate Barald and Darvin Berg. Dept. of Biology, UCSD, La Jolla, CA 92037.

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 most 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 (H)choline (10.1 Ci/mmol) followed by 1 hr in 10 μM unlabeled choline. The cultures were fixed in glutaraldehyde, post-fixed in osmium, rinsed, coated with photographic emulsion, and developed over 3-5 days.

SC 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 some of the neurons labeled. Myotubes also remain unlabeled. SC neurons prepared from 4-day embryos displayed a spectrum of labeling when grown with myotubes for 1 wk. About half (56 ± 45, 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 light microscopy. Out of 17 cells examined, 16 were clearly labeled. By comparing the number of labeled neurons in cultures incubated with (H)choline, (H)-α-amino butyric acid (GABA), or both, we concluded that the labeled cells were cholinergic. No (H)choline labeling could instead be labeled by (H)GABA, and vice versa. Thus different populations of neurons appeared to be labeled by each compound. In some cultures (H)choline labeling may not be restricted to cholinergic neurons. For example, cultures prepared with SC cells from 7-day embryos that were transfected to have many fewer cholinergic neurons than SC cultures from 4-day embryos, yet they appear to have as many (H)choline labeled neurons, some of which may also label with (H)GABA. In all cases (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 transit 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 F12861, Muscular Dystrophy Assoc., & Am. Heart.)

ULTRASTRUCTURAL CYTOCHEMISTRY OF PLASMALEUM OF CULTURED HUMAN MUSCLE. Valerie Atanasiu and F. E. Engel. NH, 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 monolayer probe described by (A (Con A) and the lectin glicotoxin, and tannic acid to describe the ultrastructural cytochemistry of the plasmalemma of normal human muscle cells in vitro displaying different stages of growth. Since both lectins have a globular pattern of peroxidase-post-coupled DAB-reaction Con A staining of the plasmalemma, indicating the presence of α-D-glucoside and/or α-D-mannose 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, as an indication of abnormalities 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. When the cultures were in 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 of cultured human muscle could have been prevented. 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 sarcolemmal plasmalemma of cultured embryonic chick and adult rat skeletal muscle showed the differential expression of plasmalemmal tannic-acid staining with muscle fiber maturity; in addition, the clearly identifiable t-tubules of the mature muscle were more distinctly stained, as were the t-tubules originating “lace” of the cultured mature embryo fibers; an outer layer of the avian leucosis-sarcoma viral C-particles in the cultured chick-embryo was also stained.)

Therefore, tannic acid good probe for studying muscle cell maturation in culture. The described cytochemical staining of normal human muscle during development now provides a basis for further plasmalemmal abnormalities in cultured abnormal human muscle.

EFFECT OF GLIAL CONDITIONED MEDIUM ON SURVIVAL AND FIBRE OUTGROWTH FROM CHICK SENSORY NEURONS. Yves A. Barde*, Ronald H.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 macromolecular termed "glial factor" which has been identified in the medium conditioned by cultures of sympathetic ganglia cells. It 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), which contains higher concentration of "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 noticeable increase in the number of surviving neurons. The effect of GCM could be completely abolished by specific antibodies to NGF. In contrast, the increased survival of neurons promoted by GCM was not blocked by antibodies. 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 conclu ded that the effect of survival induced by this procedure is due to some fibrosis of isolated sensory neurons were brought about by a factor other than that which is responsible for neuroblastoma cell growth or "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 glycoprotein. The characterization of this factor is now in progress. We are also examining the possibility that this factor might be influencing the survival of sympathetic cells in culture.

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 muscle cells from SC (Freschi et al., J. Cell. BioI. 74a, 1977). Protein content, neuronal cell count and activities of cholinesterase (ChE), glutamic acid decarboxylase and other enzymes are not similarly increased. It is not known whether this induction of CAT activity is associated with changes in other parameters of cholinergic neurotransmission. The present experiment examined the effect of skeletal muscle CM on the binding of [3H]quinuclidinyl benzilate ([3H]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 suspensions onto collagen-coated dishes at a cell density of approx. 2,500/cm². [3H]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 ± S.E.M.). Specific [3H]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 3-5 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 21-22 days. The corresponding factor for ChE specific activity at 18-21 days was 1.2±0.1 (N=3). In parallel experiments, [14C]thymidine incorporation by explants of rat embryos was 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.)


Striated muscle fibers have been observed within the pinal glands of several mammalian species including man. We found striated muscle fibers in each of twenty consecutive pinal glands cultured from individual neonatal (2 day) rats. Subsequent experiments were done with dissociated cultures of pinal glands pooled from several litters. Myocytes were first visible after about one week in culture. During the next several weeks the myocytes increased in size, developed cross-connections, 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 (ML), and -aminobutyric acid had no effect on the RMP when applied iontophoretically. The ACh response was reversibly blocked by 10⁻⁷M d-tubocurarine and irreversibly blocked by 10⁻⁵M 1-bungarotoxin. Atropine at 10⁻⁷M reduced the amplitude and shortened the time course of the ACh response, and 10⁻⁵M produced complete but reversible inhibition.

It is concluded that myogenic neurons of unknown origin occur within the neonatal pinal gland, and that these muscle fibers are electrophysiologically and pharmacologically identical with peripheral skeletal muscle cells in vitro. Although the pinal gland is devoid of ACh, these striated muscle fibers develop ACh receptors but do not develop receptors mediating electrophysiologically responses for NE, 5HT, DA, 5HT, or GABA which are known to be present in the pinal. A comparison of the developmental properties of these cells in culture with other systems such as cultured muscle fibers in dissociated thyme (Mueller, et al., Nature 256: 493-494, 1975) suggests the possibility that these pinal striated muscle fibers may arise from pluripotential stem cells.


Individual ciliary ganglion (parasympathetic) and lumbar chain (sympathetic) neurons from chick embryos are cultured 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 antisera 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 polyaniline-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 trypanin, 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.


A UV laser microscope system is being utilized to a) transplant neurites of nerve cells in culture, b) create adhesion patterns on glass or plastic surfaces and c) de-insulate and regions of photosed gold in the field of the fiberoptic microscopic chamber. 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 10² W/cm². Successful transactions depend on the power density utilized, the exact position of the focus relative to the plane of the neurite, the distance of the transaction 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. EH 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 the tungsten tip and the concomitant pressure-release of the underlying insulation. A 10 µm diameter crater in a 3 to 4 µm thick insulation layer has an impedance of approximately 3 megohms at 1 kHz. These recessed gold surfaces have recorded single unit activity from small brain ganglia resting against the recording matrix in a slow flow pool of artificial cerebrospinal fluid. Cells produce 3 µV 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 gateable to 20-25 µV spikes. Glass cells produce 3 µV 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 gateable to 20-25 µV spikes. Glass cells produce 3 µV 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 gateable to 20-25 µV spikes.

950
1893 HRP CHARACTERIZATION OF NEURONS IN ORGANIZED CULTURES OF CEREBELLUM. W. J. Hendelman and K. C. Marshall, Dept. of Anat. and Physiol., University of Ottawa, Ottawa, Canada, KIN 9A9. Organotypic (Maxnow) cultures of newborn mouse cerebellum have 3 different regions: the cortical area with its Purkinje neurons (PN), the deep cerebellar nuclear cell (DN), and a group of cells derived from the brain stem in the peduncular region (BS). Clearly identified neurons were visualized in selected mature cultures and injected with horseradish peroxidase (HRP, Sigma type VI), using depolarizing pulses equivalent to about 100-120 x 10-3 Amp.-minutes of steady current. Perfusion of the cultures (30-40 min) was continued for 4-6 hours, and followed by aldehyde fixation. The reaction in Harker-Rates solution was monitored microscopically for 2 hours by oxygen radicals generated by the general depolarization of small cells (25 or less) were not stained, though the axons were well filled. The PN axon had a constant diameter of 1-5 μ and followed a relatively direct course to the DN area. Most axons emitted a single recurrent collateral. In the BN region the axon arborized in a narrow field (appr. 60 x 150μ) and terminated in knobs (2-5μ); some axons branches had "terminals-en-passant". The BN 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 axones or along slender side branches. BS neurons: These large cells (30μ) have 2-4 dendrites which are broad at their origins and emit synapses. They taper gradually and continue to feed off axon-like neurites which may extend for some distance. Some neurons appear to give rise to multiple axons emanating from soma and dendrites. The axones are thin, and characterized by small varicocities along their course. This is usually an extensive plexus of local collaterals, but the longer branches meander erratically into the cortical and the non-neural outer granular regions. This technique has been useful to demonstrate the axonal branches of a single neuron. These projettions corroborate our electrophysiological data on the interconnections in these cultures. Further work is intended to define the synaptic connections microscopically. (Supported by the Medical Research Council of Canada).

1894 BULK ISOLATION FROM RAT CEREBRAL CORTEX OF VIABLE NEURONS WHICH RETAIN SYNAPTIC COMPLEXES. W. B. Huttner*, R. Meyermann, V. Neuhoff* and H. H. Althaus*. (ESPP: P. Greengard). Max-Planck-Institut Exp. Med., 74 Goettingen, FR Germany. 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 neurons were carried out (2). The basis of this procedure is the predissaggregation of the neurons in situ brought about by perfusion of the brain under specialized conditions: 1) a hypertonic concentration of inorganic salts; 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 predissaggregation 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 105 nerve cells/cortex. Upon SEM and TEM examination of the isolated neurons, varicosities 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 synapses on their plasma membranes. Preliminary experiments have determined that the nerve cells can be maintained in culture (Althaus, Neuhoff, Huttner, Monzai and Shahar).


2) Huttner, Meyermann, Neuhoff and Althaus, submitted for publication.

1892 SYNAPSE FORMATION BY NEUROBLASTOMA AND HYBRID CELL LINES. Haruhiko Higashida*, Steven P. Wilson, Michael Adler and Marshall Wiltner*, Laboratory of Biochemical Genetics, NHLBI, NTR, Bethesda, MD 20014.

Kineine neuroblastoma or hybrid cell lines that synthesize acetylcholine (ACh) and depleted acetylcholinesterase activity were tested for synaptic formation. Myotubes were tested for synapses by intracellular recording using the presence of spontaneous miniature endplate potentials as criteria for formation. The cholinergic parameters of these cell lines are as follows: Rates of ACh synthesis were 35-460 pmol ACh/mg min/mg homogenate. Cells incubated with 100 mM [3H]-choline ribaslated 80-4,370 × 3H-ACh/mg (Wilson, et al., Fed. Proc. 37, 1784, [1978]). Six cell lines formed synapses with high frequency (Syn+ lines) (14-63) of myotubes tested were formed rapidly, 20-30 min after cells were added to myotubes. These cells also formed synapses with clonal GI-1 mouse striated muscle cells.

Some Syn- cell lines undergo depolarization when activated by ACh, serotonin, or dopamine. Addition of neurotensin, angiotensin II, and other hormones were not effective in promoting synapse formation. The antagonists flumazenil and oxotremorine, were tested for inhibition of synapse formation and function thus can be studied. Addition of forskolin to Syn+ cell lines failed to increase the yield of synapses formed. However, forskolin increased the number of synapses formed, the frequency of spontaneous synaptic responses, and K+ concentration-dependent release of ACh from Syn+ lines. These results show that stimulus-dependent ACh release is regulated by a complex of events, thereby regulating synapse formation and efficiency, and that clonal cell lines can be generated with defects in de-polarization/ACh-releasing coupling and other presynaptic functions.

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 these 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 stage may only be partial in our cultures.

Our results show that La++ (1.4M to 3M) administration always results in decreased contraction frequency and strength with complete block of spontaneous contractility at higher concentrations. These changes, however, were paralleled by marked alterations in electrical activity. Intracellular recordings during this trend towards complete block of contractility show a concomitant progressive cell depolarization, diminished spontaneous activity, and increased action potential duration with alteration of normal configuration. Action potentials from cells showing slow rhythmic spontaneous contractile activity were diminished in duration of up to 2.5 sec. All cells which showed complete block of contractility after exposure showed resting membrane potentials which ranged from -250mV to -150mV (CA++ only) 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 saline. These results parallel the increased strength and contraction frequency paralleled by increased action potential discharge frequency and levels of membrane-polarization.

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, GM2345 and GM2754, and by U.S. Air Force AFOSR-77-3139.


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 culture system, particularly if the latter could be made available as a purified, homogenous population. Cells, suspensions were obtained from neonatal rat cerebrum by trituration after trypsin treatment. The cells were cultured on glass coverslips in CO2/saturated fetal calf serum-supplemented medium. Coverglasses were inspected periodically under phase contrast microscopy for morphology and numerical analyses, and examined at selected times for intracellular injection of horseradish peroxidase as evidence 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 (thinnly 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 weekold cultures started at appropriate seeding densities. A combined treatment of serum withdrawal and dibutyryl cyclic AMP (DBCA, 1mm) causes these cells to assume typical astroglial 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 on 2-week old confluent cultures. 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 treatment.

2) Process-bearing (PB) cells were present even as early as the cultures could be analyzed, when they equaled or outnumbered the flat cells, but showed no propensity to proliferate. Process-bearing PB were characterized by: i) small, well-contained bodies with either a phase-dark or a phase-bright appearance, ii) discrete, thin processes varying in diameter 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)

GROWTH CHARACTERISTICS OF ISOLATED ADRENAL MEDULLARY CELLS IN CULTURE. Bruce G. Livett, Daemo M. Dean* and Garth M. Bray, Division of Neurology, Montreal General Hospital and McGill University, Montreal, Quebec, Canada, B3G 1A4

Periphera 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, 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 DME 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 varicosel appearance. Examination of the cultures by the Pagi fluorescent method revealed high concentrations of catecholamines in the varicosel processes and a few varicosel-free cell processes. Transmission electron microscopy confirmed that the processes contained either adrenaline or noradrenaline vesicles. Chemical analysis of the overall cultures showed that they contained principally noradrenaline (NA) (NA/NA 1.24 ± 0.07, n=5) in contrast to the cells which, when first plated, contained mainly adrenaline (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 in vitro these chromaffin cells can undergo process formation resulting in varicose fiber networks similar to those of adrenergic neurons.

(Supported by N.R.C.)

MORPHOLOGY AND ELECTROPHYSIOLOGY OF CEREBELLAR NEURONS IN CULTURE. T. A. Meas, J. A. Hoage, I. B. Williams, R. L. Haxby, W. G. Gibbs* and P. G. Nelson. LDM, NIDs, NIH, Bethesda, MD 20014

The cerebellar cortex has been used as a model for the study of neural 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 synaptic connectivity is reduced and neurons are more readily accessible for intracellular recording and structural tracing. We have obtained, from 17-19 day fetal rat cerebellum, long (4-7 M) cell cultures which contain several types of neurons. Intact cerebella were passed through Nitex 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 some diameters 15μ. Although membrane potentials were large (>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 were the varicose round soma, one or more dendritic shafts with blunt-ended, spine laden branches, and a single axon which bifurcated several times, but showed few swellings in contact with the soma, ipsilateral. These swellings were seen to contain pleomorphic vesicles. Morphological features of such neurons suggest that they are Purkinje cell dendrites whose dendritic arborization is reduced and neurons are more readily accessible for intracellular recording and structural tracing. We have obtained, from 17-19 day fetal rat cerebellum, long (4-7 M) cell cultures which contain several types of neurons. Intact cerebella were passed through Nitex 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 some diameters 15μ. Although membrane potentials were large (>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.

MORPHOLOGY AND ELECTROPHYSIOLOGY OF CEREBELLAR NEURONS IN CULTURE. T. A. Meas, J. A. Hoage, I. B. Williams, R. L. Haxby, W. G. Gibbs* and P. G. Nelson. LDM, NIDs, NIH, Bethesda, MD 20014

The cerebellar cortex has been used as a model for the study of neural 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 synaptic connectivity is reduced and neurons are more readily accessible for intracellular recording and structural tracing. We have obtained, from 17-19 day fetal rat cerebellum, long (4-7 M) cell cultures which contain several types of neurons. Intact cerebella were passed through Nitex 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 some diameters 15μ. Although membrane potentials were large (>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.

Sodium purines and pyrimidines, including ATP and UDP, produce potent changes in cultured bullfrog sympathetic neurons which are similar to cholinergic muscarinic responses (D'Angioni et al., Science 263,1977). In the present study we have analyzed the responses of these neurons to muscarine, a cholinerogic agonist thought to act primarily at the muscarinic receptor, and to nucleotides. The muscarinic components of responses to long-term (4-8 weeks) cultures of adult bullfrog sympathetic ganglia were studied using conventional intracellular recording techniques and superfusion of drugs (Padjen et al., Neurosci a 26,1977). In the present study we have analyzed the responses of these neurons to muscarine, a cholinerogic agonist thought to act primarily at the muscarinic receptor, and to nucleotides. The muscarinic components of responses to long-term (4-8 weeks) cultures of adult bullfrog sympathetic ganglia were studied using conventional intracellular recording techniques and superfusion of drugs (Padjen et al., Neurosci a 26,1977). The results show that nucleotides activate the same muscarinic receptors as muscarine and that the nucleotide responses are not due to contamination by muscarine or its metabolites.

MORPHOLOGY AND ELECTROPHYSIOLOGY OF DISASSOCIATED MOUSE HIPPOCAMPAL CULTURES. J. Peacock, D. Rush, and L. Mathers Stanford University Medical School, Stanford, CA 94305

Disassociated hippocampal cultures fom 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 branching but difficult to see with phase contrast optics due to their growth among underlying non-neuronal cells. They could not be identified by phase contrast staining of silver or intracellular injection of the fluorescent dye Lucifer Yellow. Some processes appeared to have spines and others to be extensively branched. Processes terminated in the soma of 22/28 fluorescent-stained cells either originating at the cell body (11 cells) or from a single trunk (11 cells). Commonly, there were 3-4 and up to 6 orders of branches or spines on neurons. They were found up to 200 cells with K-accetate or Lucifer Yellow filled micropettes. Repetitively firing APs were elicited by intracellular stimulation and frequently were preceded by stereotypic prepotentials of which the sites of origin were processes remote from the cell body. In some cells, the AP could have depolarizing prepotentials (0.3-2 sec) and superimposed action potentials. APs in these highly electrically active cells were short (0.6-1.2 ms) with peak rates of 100-150 per sec from 320-1200 Hz per 24 cells), and corresponding rates of fall from 107-677 v/s (mean 351 ± 167 v/s/24 cells). Following the AP, the hyperpolarization was usually prolonged by 30-70 ms), but postburst hyperpolarization could last < 2.5 sec.

Synapse formation was demonstrated in the following ways: by spontaneous occurrence of PSPs which could be quantified by consecutive cells or excitatory leading to APs; by reversal of inhibitory PSPs (Vrev = -40 mV); by the detection of spontaneously coupled cell pairs; and by ultrastructural identification of synaptic junctions. Further evidence for the occurrence of contacts on single processes with a predominance of symmetric over asymmetric junctions. Neuronal networks formed in these cultures demonstrated reciprocal innervation (3/19 synaptic pairs) and multiple innervation (2/19 pairs). Widespread synchronous bursting could occur among all cells, including bursting (up to 25 cells per microscopic field have been found).

These experiments show that a well-developed branching morphology of cultures of hippocampal neurons not infected by virus are found in hippocampal neurons which have been grown in culture for 1-2 months. (Supported by NIH Grant NS12151.)

Monolayer cultures of isolated sympathetic neurons and skeletal myocytes were grown in diffusion culture. Toxin-containing supernatant from cultures of 3-4 day-old chick embryos was added to the cultures of 2 day-old chick embryonic pectoral muscle and plated onto collagen-coated coverslips (200 cells/cm²). f-7 days post to the addition of the neurons, neuron-myoocyte pairs were observed with intracellular microelectrodes for synaptic interaction. In 27 of 75 pairs tested an action potential evoked in a neuron by an intracellular depolarizing current pulse gave rise after a latency [range 2 to 20 msec] to an excitatory junction potential (EJP) in a myocyte. The amplitudes of the EJPs ranged from 3 to 25 mV. In the few cases tested, the EJPs were blocked by d-tubocurarine (1 μg/ml) and a-bungarotoxin (5 × 10⁻⁷ M). The effect of these agents on the neuron-myoocyte interaction suggests that transmission was nicotinic-cholinergic. In a few experiments neuron-myoocyte pairs in the cultures were treated with intracellular microelectrodes for synaptic interaction, the characteristics of this interaction are presently under investigation. Supported by USPHS Grants NS12901, 5-B01RR07009-12, and a Muscular Dystrophy Association postdoctoral fellowship to B.F.

1904 α-BUNGAROTOXIN BINDING SITES AND ACETYLCOLINE RECEPTORS IN CHICK CILIARY CILIARIS GANGLION CELLS IN CULTURE. Peter Raybun*, Ralph Mitkin, and Barzin Berg. Dep. of Biology, UCSD, La Jolla, CA 92039.

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-containing supernatant from 2-day-old chick ciliary ganglia was grown for 1-2 weeks in cell culture with chick skeletal myocytes. Tetramethyl rhodamine-conjugated α-Bgt was used to localize the distribution of toxin binding sites in ciliary ganglia and relative 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 neuronal processes were labelled with α-Bgt. The labelling of individual cell bodies was generally uniform with occasional small clusters appearing on a few of the neurons. The range in intensity of toxin binding was similar in different neurons in many of the culture dishes; some apparently morphologically normal neurons had no detectable labelling, while others in the intermediate intensity were evident. Fluorescent toxin binding could be blocked by 140 μM-d-tubocurarine or excess unlabelled toxin. Prelabel with 0.1 μM α-Bgt blocked most of the fluorescent toxin, suggesting that the sites had been largely saturated. [125I]α-Bgt binding studies indicated a mean site density of ca. 10⁶ per neuron.

The neurons showed a wide variability in their sensitivities to parasympathetic applied ACh even after normalization for differences in resting potential (mean: 51 mV) and input impedance (mean: 45 MΩ). Precubation 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 myocytes but caused no significant change in the sensitivity of the neurons. The sensitivity for neurons in control cultures was 104.27±2.18 (SEM=23, n=18) and in toxin treated cultures was 82 μM/EC50 (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 (Cappiello et al., PNAS 75: 1016 (1978)) and a neuroblastoma 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 myocytes. (Supported by USPHS Grant #12601, The Muscular Dystrophy Association, and The American Heart Association.)

1906 IN VITRO HUMAN NAVALMAI PACEMAKER NEURONS. Michael S. Raybourn and Cornelius A. O'Leary, Radiation Biology Group, Homer Lab/LLNL, Univ. of California, Berkeley, Berkeley, Ca. 94720

We are currently investigating the cellular biosyntheses of neural 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-P). These cells presumably derive their drive force from their spontaneously-mediated inputs. The rest of the Purkinje cells revert to their normal stochastic firing patterns to a clock-like pacemaker (P) rhythm. 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. P, non-P) 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 hypoxia and carbon monoxide insults. These effects are generally phototaxically correlated and we are currently attempting to determine the action spectra for this in order to estimate the site of direct cellular activity. In addition, we are using the protein synthesis inhibitor, Actinomycin, in order to ascertain what role, if any, that protein synthesis might play in determining the endogenous activity of pacemaker Purkinje cells.
1907

**Pineal Cells Enhance Choline Acetyltransferase 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 64110.

Monolayer cultures of cells derived from neonatal 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 2.56 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 nmoles/dish/hour). CAT activity in the cocultures, however, was up to 10 times that observed in the SCG's cultured alone (180 vs. 18 nmoles/mg protein/min).

Pineal cultures 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 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


The electrical properties of human neurons have been characterized quantitatively through an extensive intracellular electrophysiological investigation of cell cultures of dorsal root ganglia (DRG). In three cases, cultures were prepared from fetal DRG (13, 14, and 27 weeks gestation) and from neonatal and young adult (3 months post-natal) DRG. 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 DMEM-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 K) were transfected to 4mM K and the following features examined: resting membrane potential (Vm); cell input resistance (Ri), specific membrane resistance (Rm), rheobase (Ir), voltage change at rheobase (Vm-Ir x Ri), duration of the action potential (AFT), time constant (TC, determined from strength duration data), and absolute refractory period (ARP).

The table below illustrates the results obtained from four specimens grouped according to the K concentration of the culture medium.

<table>
<thead>
<tr>
<th>K</th>
<th>Vm*n</th>
<th>Rl*n</th>
<th>Rm</th>
<th>Ith*n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nM)</td>
<td>(mV)</td>
<td>(M)</td>
<td>(mV)</td>
<td>(nA)</td>
</tr>
<tr>
<td>0</td>
<td>48.7±7.9(222)</td>
<td>22.5±7.9(178)</td>
<td>1074±391(176)</td>
<td>0.65±0.03(187)</td>
</tr>
<tr>
<td>4</td>
<td>52.7±6.0(307)</td>
<td>45.1±1.0(205)</td>
<td>1039±462(202)</td>
<td>0.91±0.04(206)</td>
</tr>
</tbody>
</table>

1909

**Proliferation of Glial Cells in Continuous Cell Cultures Following Kainic Acid Lesions of Rat Striatum.** V.K. Singh, E.M. Bohm*, and D. Van Alstyne*. Immunology Unit, Children's Hospital, Vancouver, B.C. V5K 1K2, Canada.

Glia cell grow to confluence 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 moles of kainic acid into caudate-putamen nucleus (Singh et al., Brain Res. 187:1978), 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 fibillary acidic protein (GFAP) localized through immunofluorescent and immunocytochemical 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 Benefit of Canada).

1910


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 disassembled phosphatide (DPF) treated culture medium, can be stimulated 6-10× by 5×10⁻⁷M of the calcium ionophore A23187 and can be inhibited by the calcium ionophore A23187 with transport nonmonovalent cations. For example, release of AChE is inhibited 50× by 4×10⁻⁷M Nomenanin, or 1.6×10⁻⁷M Nigenin or 7.5 ×10⁻⁷M K537A (Univ. of Conn. Med. School, 1978). Parallel 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 reversed within 2-4 hours after removal of the drug.

The rate of appearance of cell surface acetylcholine receptors (AChRs) (measured as the rate of appearance of [112] pentabutoxytoxin binding sites) is unchanged by concentrations of ionophore which maximally inhibit AChE release. Experiments employing AChE histochemistry and electron microscopy reveal intracellular microvesicle vesicles in ionophore treated cells which stain heavily for AChE. Further, the AChE of ionophore treated cells is less accessible to degradation by Nα-bungarotoxin. Preliminary cell fractionation experiments indicate that the AChE which accumulates in ionophore treated muscle cells can be found on membranes that are distinctively different from plasma membranes. These experiments suggest that the AChE accumulates on intracellular membranes in ionophore treated cells which are inaccessible to externally applied agents, while the acetylcholine receptor proceeds normally to the plasma membrane and remains accessible to externally applied AChE toxins. 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.
MODULATION of cAMP-INDUCED PROTEIN FORMATION AND ConA-INDUCED CAP FORMATION IN CULTURED GLIOMA CELL LINES. Barry H. Smith, Theodore M. Liscitzky*, Rogers Plessant*, and Paul L. Kornbluth*. Neurosurgical Service, Massachusetts General Hospital, Boston, MA 02110. The molecular mechanisms of cyclic AMP-induced protein formation, as well as the ConA-induced protein formation have been studied in a cultured human glioma cell line (M1) and 4 clones derived therefrom.  

The parent of this line, as well as all derived clones, form processes in the presence of 10^-5 M dibutyl-cAMP, although the percentage of cells exhibiting such processes varies from clone to clone (i.e., from 50% of the parent at 48 h to 80% at 90 h). 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 (lidocaine 10^-4M; lidocaine 10^-3M, and prilocaine 10^-3M) reduced the extent of ConA-induc ed microfilament-active blocks, this process formation with dibucaine being the most effective (-100%). The microtubule-blocked cell lines developed surprisingly long microtubule bundles but cytochalasin B (12.5 µg/ml), which interferes with microfilament function, results in very short, highly-branched processes, suggesting tubulin is an essential component for this type of process formation. Protein synthesis, as evidenced by the addition of 10^-5 M puromycin, is not required for process formation. Metabolic inhibition (Na azide, 10^-3M), although it reduces the total number of cells as well as the proportion of cells with processes, allows this process formation to occur. 10^-3 M Ca++ in the medium results in the formation of extremely long processes. Thromodorine inhibitors (theophylline, 10^-3M) enhance the dibucaine-induced process formation when added with dibucaine-cAMP. Papaverine alone produces bipolar cells with extremely long, wide processes, whereas theophylline alone with Con A does not result in such movements. Surface-bound molecules were monitored by binding with Con A (5-100µg/ml) which reliably produces patching and capping in this glioma line. Formation of the Con A cap was followed by light, scanning, and transmission (electron microscopy). Microfilaments (40-60Å) appear necessary for cap formation and internalization, although perhaps by virtue of a role in stabilizing the cytoskeleton, may be inhibitory. These postulates are supported by the fact that dibucaine (10^-3M) blocks cap formation as does dibucaine cAMP, which has been associated with microfilament 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. For well-coordinated cap formation out of cell lines, although the two processes differ in their mechanisms.

NEURONAL SURVIVAL AND CAT ACTIVITY IN DISOCIATED CELL CULTURES OF CILIARY GANGLION. Jeremy Tuttle*, March Ard*, and Janusz Suszke*, (apron: J. Alantia). 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. Cat cell cultures were prepared from ganglia dissected at embryonic stage 29-32. The effect upon subsequent cell survival was determined for the following three variables: 1) Plating density. No effect of plating density was noted when 4000 and 30000 cells were plated on cover glasses in 0.5 ml of culture medium. 2) Basic medium. Cell growth was most rapid when supplemented with 20% heat-inactivated horse serum (HIS), 10% FCS (fetal calf serum) and human serum (from a single donor and pooled), (10% V/V), were all unsatisfactory, for survival at 2-4 days. In cultures for long-term survival, 10% HIS cell culture in supplemented HIS without serum could support 302 weeks survival for three weeks in culture. At 1X CEE, without serum, all cells died in 5 days. When the CEE was combined with 10% HIS, up to 90% of the neu rons plated could survive these conditions. When 10% HIS was used, the majority of plated neurons could survive at least one month in the absence of serum, or in fresh 10XHISCEM (human), but only 10% remained at 3 weeks in conditioned media. Serum treated best when grown on collagen along with myoblasts, for >80% survived at least one month. The choline-acetyltransferase (CAT) activity in these cultures was also determined over three weeks in culture. CAT activity per cell increased over the first two weeks in culture both with and without myoblasts. Cultures conditioned with normal serum showed a marked but lesser rise in CAT activity. This activity in conditioned cell culture differs from that of in vivo as follows: 1) In vitro, the time required to reach an active level is longer. 2) In vivo, the rise of CAT activity is continuous, while in vitro it is not. 3) In vivo, the effect of drugs is more pronounced in vitro than in vivo. 4) In vitro, the effect is more pronounced in vitro. This may reflect a period of recovery after dissociation. 5) In vivo, the rise of CAT activity continues past embryonic stage 32 to much higher levels, whereas in vitro the activity in culture is followed by a slower increase or drop, depending upon the culture substrate used. These results suggest: 1) Some component is critical for survival of dissociated 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-NI01338, NS8382 and The Univ. of Conn. Research Foundation.

ELECTROPHYSIOLOGICAL PROPERTIES OF ISOLATED SYMPATHETIC NEUROFIBER DEVELOPING IN MICROCULTURES. Trisha Suppe and Paul R. O'Lague. Dept. Anatomy and Physiology, UCLA, Los Angeles, CA 90024. Intracellular microelectrodes were used to study the electrophysiological properties of sympathetic neurons developing in microcultures. Physiological properties of cell cultures (made of newly isolated sympathetic neurons and ganglia in diameter) islands of cardiac myocytes and one or a few (5) neurons isolated from mechanically dissociated superior cervical ganglia (SCG) were cultured on coverslips (N=20 for each), (PAMS 73: 1425, 1976). 18-90 days after planting the neurons, their electrophysiological properties were examined during continuous perfusion of the cultures (see above for reference method and perfusion medium); all neurons tested (57%) had resting potential in the range of -55 to -70 mV, action potential amplitudes of 65 to 90 mV, and maximum rates of rise of 200 to 450 V/sec. These values are comparable to those reported by others for sympathetic cultures (O'Lague, P.H., Potter, D.D., and E. J. Purashen, Devol. Biol., in press) and for sympathetic neurons in superior cervical ganglia of adult rats (Ard et al., Pfluigers Arch. 414, 1970). The ionic basis of the action potential in the neurons was studied with the pharmacological agents tetrodotoxin (TX;), a blocker of voltage-sensitive Na+ channels, and Co++ and Mn++, both blockers of voltage-sensitive Ca++ channels. TX (0.5-3 µM) 40 cases) 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 a sufficient depolarization. This all-or-none response was dependent on extracellular Ca++ (10- 20 mV per 10 fold change), was independent of changes in extracellular Na+ and abortive on Mn++ or Co++ (2-5mM). These results suggest that Na+ and Ca++ carry inward current during the action potential. In many neurons, usually in older cultures (4-8 days), a late potential was observed. For evidence for this is: 1) the LAT was accompanied by a substantial decrease in membrane resistance (in some cases up to 30%); 2) it reversed at the same potential; and 3) it was only reduced in amplitude and duration by lowering the extracellular Ca++ or by addition of Co++ and Mn++ at the same concentrations which block the action potential in the cells. The single cell neuron the expression of the above electrophysiological properties changes as it develops in culture, and whether the electric activity affects this expression are presently under investigation. Supported by USPHS Grant NS12901.
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 School 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°C in an atmosphere of 5% CO₂ - 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 3 X 10⁶ 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)

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 (H3) 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
TROPHIC FUNCTIONS

1917

ADVERSE EFFECTS OF TETRODOTOXIN ON EARLY DEVELOPMENT AND
SURVIVAL OF POSTSYNAPTIC CELLS IN SPINAL CORD CULTURES.
Laboratory of Development Neurobiology, NI CHD, 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) ce lls and spinal
cord (SC) ce lls in culture are ea sily distinguished morphologi­
ca lly and electrophysiologically. Intra ce llu la r recordings show
that SC ce lls commonly exhibit ongoing action potentials and
postsynaptic potentials, whereas DRG c e lls, while e le c tric a lly
excitable and capable of establishing synapses with SC ce lls,
are e le c tric a lly 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 inpervationExposing combined SC-DRG cell cultures to 10-6M or 10-7M
tetrodotoxin (TTX), a specific blocker of sodium conductance
channels in neurons, completely blocked a ll spontaneous e le c tri­
cal a ctivity. 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 ce lls
larger than 30μ were reduced in number to less than 9% of
controls (p<.001) while DRG cell counts were not sig n ific a n tly
different from controls. I f treatment with TTX was delayed
until 10 weeks in culture no sign ifica n t diminution of either SC
or DRG cell counts was evident. Tetrodotoxin treatment did not
affect the electrical characteristics of the DRG c e lls; resting
membrane potentials and input resistances did not d iffe r from
the control values. The remaining SC c e lls were fra g ile and
had lower resting membrane potentials than normal, but some
surviving SC c e lls revealed evidence of synapse formation in
TTX with both I PSPs 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 sp e c ific ity 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 a c tiv ity
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 a c tiv ity play crucial roles in central neuronal
development during early stages of differentiation and synapse
formation; at later states neuronal survival is less affected
bv blockade of electrical activity.

1918

RAPID LOSS OF JUNCTIONAL ACETYLCHOLINE RECEPTORS AFTER DENERVA­
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 af­
ter denervation.
In this study we examined junctional AChR after
denervation of rat diaphragm and extensor digitorum longus (EDL)
muscles by measuring the binding of (125I)α -bungarotoxin. Junc­
tional 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 dis­
sected 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 est­
imated on the basis of the extrajunctional AChR found in the im­
mediately 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 sig­
nificantly (P = 0.01) decreased by day 5 and at 7-9 days after de­
nervation were reduced to 581 of the innervated level. EDL mus­
cles were denervated unilaterally and 7-8 days later AChR in de­
nervated 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 μ g/ml (125I)
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 radio­
active 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 junction­
al 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 dif­
ference 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 ELEC­
TRICAL 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 sen­
sitivity) are regulated by the contractile activity of the muscle.
The aim of this study is to identify the mechanism by which elec­
trical stimulation regulates ACh receptor metabolism. We have
measured the effect of electrical stimulation on the synthesis
(appearance in the extrajunctional muscle membrane) of ACh re­
ceptors and their subsequent degradation (or removal) from mem­
branes of denervated rat skeletal muscles maintained in organ
culture. ACh receptors were measured by specific and irreversi­
ble binding with radioactive α-bungarotoxin.
The rate of de novo biosynthesis was measured by determining
the rate of appearance of 2H, 13C, 15N-containing ACh receptors
when muscles were cultured in medium containing 2H, 13C, l5N-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 corres­
ponding decline in overall protein synthesis. Stimulation beyond
18-24 hr (up to 68 hr) resulted in a further decrease in new re­
ceptor production to about 30% of control rate, but no more.
Stimulation for longer than 16 hr produced less than 5-10% de­
crease in overall protein synthesis compared with control muscles.
The degradation rate of extrajunctional ACh receptors was es­
timated 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 pro­
ducts of the radioactive α-bungarotoxin. The rate of this pro­
teolytic process which reflects the average ACh receptor lifetime
was 22 hr. Electrical stimulation at 100 Hz for 1 sec was ap­
plied 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 con­
tractions, 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,
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 out­
growth, 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 antibodyresistant mechanism. To determine whether E14 and NB ganglion
neurons are intrinsically different, ganglia were trypsindissociated 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.)

601


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-extend for digitonin-solubilized (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 maintained in culture for 6 days. 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 pretreatment of the block with Ht (0.1 mM), a known blocker of NMDA receptors. 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, (2) that the factor has a preferential effect on end-plate CHE, 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 CHE 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 butyrylcholinesterase, and which significantly (P<0.05) higher (20-30%) than that of muscles cultured in the corresponding control medium. (Supported by USPHS NIH grant NS 13027.)


In order to determine the approximate molecular weight of the factor, the conditioned medium was filtered with Pellicon molecular filters PTFE or PDC (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 or medium conditioned in the absence of 10% fetal calf serum was effective in reducing non-neuronal cell outgrowth. Twenty four hours after explantation, medium previously conditioned 3 to 4 days by dissociated fetal limb muscle injection into similar sized limb contralateral to the side. The muscle membrane was significantly depolarized (4-8 min) at the endplate at 30 min after BTX injection at close site; distant site required about 45 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 normal stimulation was 0.70 ± 0.11 uoles acetylcholine hydrolyzed per min per g muscle. Virtually all of this increase was due to an increase in CHE 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 CHE we determined segments 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 increased CHE activity dramatically (102% and highly significant P<0.001) in end-plate but had essentially no effect on background CHE. Cholinesterase (CHE) activity of organ-cultured EDL muscles was determined to the extent to which the factor released by neural stimulation promoted CHE synthesis. Any hexamethonium decreased the CHE 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 this factor continued to have an CHE 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.)


During experiments designed to study interneuronal neurons in the abducens nucleus (Abd.n.), it was observed in Nissl material that either intrinsic or peripheral section of the VIth nerve did not produce retrograde chromatolysis and degeneration of Abd. motoneurons (Nelken's criterion). However, atrophy, which may result in a low activity of Abd.n. from onset of axotomy to reinnervation. The results complement, but more often contrast with, the classical criteria. Atrophy was produced by axotomy. Stimulating electrodes were placed bilateral intracranially on the Vih nerve. The magnetic search coil technique was used to record eye movement. In two experiments tango microelectrodes were implanted in the Abd.n. to chronically record antidromically-elicited field potentials following axotomy. 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 decreased. Intracellular recording from Abd. n. exhibited the presence of an H spike; however, unless the cell was depolarized, 15-25% (distant site) or raphe muscle, the normal transmitter release was still maintained. The maximal effect of the conditioned medium on percent stimulus was 50%, but it never reached control level. In acute electrophysiological experiments carried out 10 days after axotomy, the antidromic field potential was also decreased. Intracellular recording from Abd. n. exhibited the presence of an H spike; however, unless the cell was depolarized, 15-25% (distant site) or raphe muscle, the normal transmitter release was still maintained. The maximal effect of the conditioned medium on percent stimulus was 50%, but it never reached control level.
1925 NEUROTROPHIC CONTROL OF CHLORIDE CONDUCTANCE IN CULTURED MUSCLE. J. K. Engelhardt, K. Ishikawa*, and Y. Shimabukuro*. Grant Neuroscience Laboratory, Div 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 \( C_m \) unchanged (Engelhardt et al., Brain Research 126: 172-175, 1977). The product \( R_m \times C_m \) is equal to the electrical time constant \( \tau_m \) of the muscle fiber; therefore, \( \tau_m \) measurements can be used to estimate membrane capacitance \( C_m = R_m / \tau_m \). When \( \tau_m \) 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:

\[
\begin{align*}
G_m (\text{Normal Cl}^-) &= G_X + G_{Cl} - G_K \\
G_m (\text{Low Cl}^-) &= G_X + G_{Cl} - G_K
\end{align*}
\]

where \( G_X \) is the conductance due to chloride ions.

When the product \( R_m \times C_m \) is increased from pure muscle cultures are compared with those from muscles that have been co-cultured with cord explants, the above assumptions result in the conductance values (in \( \mu \text{siemens/cm}^2 \) tabulated below:

\[
\begin{align*}
R_m &/ C_m \\
\text{Pure Muscle} &| 575 | 510 | 65 \\
\text{Nerve + Muscle} &| 603 | 563 | 67
\end{align*}
\]

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 (ALSSA), The Myasthenia Gravis Foundation, and The Wright Foundation.)


The mechanism(s) underlying neurotrophic 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 the 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 gracliius muscle (short stump) or 3.5-4cm central to that point (long stump), leaving the contralateral nerve intact (control). At varying postoperative times gracliius 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 by from 2D acrylamide gels to sucrose gradients. Endplate enriched zones of control gracliius contained 16,10 and 4S AChE, whereas those from F72 control preparations, the source of ADP-glucose (L-Moss et al., J. Neurocytol. 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).


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 the uptake of radiochemical. Under these conditions, however, was different; enzymatic decay started considerably earlier in short stump (3-4d) preparations. 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, the effects of protein inhibition, the protein counts for areas overlying the tissues were reduced to background. Thus we have observed similar activity in complexus ("hatching") muscles of normal chicks at 5 days after "chase". "Insertional" activity was elicited from pectinal 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 chick 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 after "chase" (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 complexus muscles is the hypertrophy of fibers, on days 16-20 in vivo, followed by atrophy until day 7 ex vivo (Ashmore et al., J. Histochem Cytochem. 21:246-271, 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.)


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 after "chase". "Insertional" activity was elicited from pectinal 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 chick 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 after "chase" (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 complexus muscles is the hypertrophy of fibers, on days 16-20 in vivo, followed by atrophy until day 7 ex vivo (Ashmore et al., J. Histochem Cytochem. 21:246-278, 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.)
ALTERATIONS IN THE IN VITRO PHOSPHORYLATION OF NUCLEAR PROTEINS AFTER DENERVATION OF SKELETAL MUSCLES. Irene A. Held and Rock C. Yeh. Neuroscience Research Laboratory, VA Hospital, Nimes, IL 60151 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 50µM Tris:HCl, pH 7.5, 0.1M NaCl, 2mM MgCl2, 20 µCI [32P] - γ -ATP and Lam cyclic AMP, when added. After termination of the reaction with excess, cold ATP and Na₃P₀₄, aliquots are taken for determination of the specific activity of endogenous protein kinase activities by measuring the incorporation of [32P] radioactivity on gel slices. The phosphorylation profile also differs. At least five of the major protein bands of soleus nuclear proteins have high levels of γ -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 radioactivity on gel slices. No change is observed in the protein pattern after denervation.

ALTERATIONS IN THE IN VITRO PHOSPHORYLATION OF NUCLEAR PROTEINS AFTER DENERVATION OF SKELETAL MUSCLE NUCLEI WITH CYCLIC AMP ARE BEING STUDIED. The factors responsible for the mediation of the observed alterations in the phosphorylation of proteins after denervation of skeletal muscle nuclei remain unidentified. Supported by NICDS Grant NS-11755 and by the Medical Research Service of the Veterans Administration.

Nerve growth factor (NGF) is present in high concentrations in the salivary glands of dystrophic mice than in glands from normal animals; glands from male dystrophies contain less NGF. Concentrations of epidermal growth factor (EGF) measured by RIA are reduced by amounts comparable to that for NGF. On the other hand, NGF, however, are not altered in the afflicted animals of either sex. Furthermore, the deficit in the glands is not limited to gland, is normal in dystrophic animals. Thyroid follicles in the treated embryos showed a general reduction 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 the mesencephalic nucleus of the trigeminal nerve. Supported by NIH grant NS03915.

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 (300,000-600,000 daltons) and it does not dissociate at low concentrations (mg/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.

The 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 detected by radioimmunoassay is not NGF but rather an immunologically 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 interfere 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.


Some 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 placental serum and chick embryo extract. 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 placental serum and chick embryo extract. 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 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 postjunctional receptors.
VESTIBULAR SYSTEM

Vestibular reflexes involving the neck musculature play an important role in controlling posture and mediating eye-head coordinations. Due to the mechanical limits of the head and neck, one important aspect is for the control of head position in the vertical plane. Therefore, as part of our efforts at determining the dynamic characteristics of the vestibulo-nuchal reflex, 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 examined since the biventer cervicus either exhibited a significant response (i.e., during roll) or antagonistically (during pitch) and therefore might demonstrate the reciprocal organization of vestibular reflexes in this region of the autonomic nervous system.

Alert cats, whose heads and spinal vertebrae (C7-8) were rigidly fixed, were subjected to sinusoidal rotations in the frequency range of 0.1 to 4.0 Hz. In this way, we measured the location of the tonic activity in the biventer cervicus which, if the head were free to move, would have served 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 they became less: about 120 degrees at 1.0 Hz and only 50-85 degrees at 4.0 Hz. Since the sternomastoid muscle was usually silent in the alert state, 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 cervicus.

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 become 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 canal and otolith inputs, there is likely to be a significant contribution to the motor outputs by the direct pathways at the higher frequencies. This is most probably necessary to present 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.


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 previously examined how information converges on single cells in the vestibular nuclei in paralyzed and anesthesitized rabbits. Rabbits were mounted with their heads placed near a post-center of a balance table, allowing sinusoidally oscillated about the vertical axis activating the horizontal semicircular canals, and about the longitudinal axis, activating the anterior and posterior semicircular canals. Visual optokinetic stimulation in the horizontal and vertical planes was delivered by projecting a random dot pattern off two mirrors mounted on a monocular and onto a rear projection screen subtending 72 x 72 deg. All vestibular units tested evoked both a vestibular and visual responsiveness. Most cells which were primarily sensitive to rotation in the horizontal 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 (0.01-0.02 Hz), as well as at higher frequencies of stimulation (0.05-0.8 Hz); indicating that visually responsive cells receive convergent otolithic and semicircular canal afferents. 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 neurons 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 ablated by ipsilateral total type I vestibular nuclei neurons, reduce the overall level of activity of these cells but do not abolish their visual directional selectivity. The immediate visual responsiveness of vestibular nuclei neurons originates from extracellular sources. (Supported by NIH grant NS-13889 to RLG.)

1939 VESTIBULO-SPINAL SYSTEM ADAPTATION TO SUDDEN UNILATERAL VESTIBULAR LESIONS. P. Owen Black and Conrad Wall III. Department of Otolaryngology, Eye and Ear Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, USA.

Commonal disorders with unilateral vestibularedisturbances 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 estimate and analyze patients' posture 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
1940

Investigations of the rostral projections from the vestibular system in the alert monkey by evoked potential techniques have revealed that activity originating in the vestibular nerve reaches the ventral basal (VB) and posterior (PO) nuclei of the thalamus. In this study, single units in VB and PO were activated by both vestibular and visual stimuli. Seventy-three of 331 units recorded were responsive to these sensory stimuli. Units that were activated at short latency (<30 msec) following vestibular nerve stimulation or with receptive fields confined to the contralateral body surface were termed "somatic non-convergent units." These units were found preferentially in VB. Units responding exclusively to auditory and somatosensory non-convergent units (4% of responsive units) were found in the medial geniculate nucleus. A third class of units were termed "convergent" (23% of responsive units). These units either: 1) responded to auditory and somatic stimuli; 2) responded to vestibular nerve stimulation at short latency (<30 msec); or 3) had a bilateral receptive field. Convergent units were found throughout PO and along the border between PO and the thalamus.

(VSided by USPHS Grant NS 11307)

1942
VESTIBULAR CORTEX IN THE ALERT MONKEY: NEURONAL ACTIVITY IN AREA 2v DURING ROTATION IN THE DARK AND OPTOKINETIC STIMULATION. U. Buttner*, U. W. Buttnerr*, and V. Henn* (ION: T. T. Kennedy). Dept. of Neurology, University of Zurich, 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 the 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 animal, was rotated sinusoidally at frequencies between 0.01 and 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 to the opposite side. The remaining half were type II neurons with a mirror-like behavior. At frequencies between 0.1 and 1 Hz, optokinetic nerve stimulation showed a phase advance of 90° 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 a phase analysis of the responses the cylinder, or the turntable, had to move in opposite directions, which elicits nystagmus in the same direction. The response to optokinetic stimulation was generally synchronous and nearly equal in the two hemifields. It was noticed that neurons still responded at high frequencies (0.5-1 Hz) with small eye displacement.

Supported by Swiss Nat. Foundation (5,672.77) and Deutsche Forschungsgemeinschaft (Bu 379/2).

1943

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 non-linear responses, the requirements for linear analysis of the data cannot be met satisfactorily. Also, the experimental conditions that would allow for the linearization of such non-linear responses are extremely difficult to achieve for this type of vestibular stimulation (French, A.S., et. al., Kybernet. 67(1972), 15-23; Gardner, E.P. and Fuchs, A.F., J. Neurophysiol. 31, 942-950; Spekreijse, H. and Oosting, H., Kybernet. 8(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 each of the stimulus patterns listed in the figure. It is a matrix whose resolution is finer than 50 msec would be required in order to distinguish the different patterns.

Programs have been written for the LINC-8 Computer to calculate these distances. These algorithms are also utilized to compute a new pattern that is closest to the given pattern among a collection of observed firing times, i.e., a kind of "typical" pattern.

Supported by NIH Grant #RR 00165 and NASA Grant #NSR 11-001-045.

1943

Many neurons in the cat vestibular nuclei which are sensitive to linear acceleration were found to be strongly influenced by moving visual stimuli in the dark. These two inputs provide congruent information about the cat's self-motion, and it can be concluded that the vestibulo-ocular reflex is produced in conditions resembling those studied here. Within-unit analyses of phase relationships showed minimal changes in phase under these experimental conditions that would allow for the linearization of the non-linear responses the vestibular inputs about self-motion do not combine additively. These two inputs provide congruent information about the cat's self-motion.

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 1 mm from the labyrinthine sensory epithelium. 200 units were studied, following each unit's spontaneous 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 in 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 decrementing frequencies applied directly to the recorded nerve fiber. These currents almost certainly modulate frequency directly by flowing in an antidromic direction thus the afferent fiber into the extracellular space. Changes in frequency are also produced by currents passed across the amputated wall. As transmission between Type II hair cells and primary afferents is chemical, lumino positive or negative transpilomy need not act directly on presynaptic secretory membrane to increase or decrease transmitter release respectively, thus modulating discharge frequency orthodromically. In regular unit changes in firing frequency of APs and H-DSs are seen 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 highly branched dendrites which would be subject to ongoing transmitter release from numerous hair cells simultaneously. This electrotonically remote depolarization will be additive and necessary towards the facilitation region leading to 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 state, ion currents passed directly into the nerve are presumably acting on this same spike initiation region to affect spontaneous frequency.
1948 DIFFERENTIAL RESPONSE OF THE CRISTA TO SINEUSOIDAL ROTATIONAL. 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 asymmetric 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 amputal nerve fibers to also show response characteristics that are dependent on the position of their associated receptors along the length of the crista. In this study, electrophysiological characteristics of single unit afferent fibers from bullfrog horizontal amputal nerve were examined and correlated with the spatial distribution of their innervation. Single fibers were dissected from the surface of the main nerve bundle with steel hook electrodes and their afferent potentials recorded during sinusoidal rotational movement (rotation amplitude: 0°-40°; frequency: 2.0-8.0 Hz). Fibers were classified as belonging to one of three groups according to their projection into the crista. The first group innervated the thin portion of the crista, adjacent to the narrow end of the crista, adjacent to the narrow 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 threshold, 3) peak firing rates that reached a maximum and then fell 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 thin portion of the cupula, the second group innervated a second region of the crista, and a third group innervated the broad end. Fibers that projected to the thin portion of the cupula also showed response characteristics that are dependent on the position of their associated receptors along the length of the crista. It is concluded that separate channels of vestibular input are preserved which are not available to the unadapted subject.

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*, Aviation Medical Research Unit, Dept. of Physiology, 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 occluded 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 +10° 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 +145° 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.


The lateral vestibular nucleus (LVN) is the origin of the lateral vestibular limb of the vestibulospinal tract. It has been extensively studied neuroanatomically and neurophysiologically. Lesions in LVN produce severe deficits in tests of posture and movement in adult rats (Modiano et al. J. Neurophysiol. 36: 1031, 1976) and neonatal rats (Tomar and Korner, Dev. Psychobiol. 1976). They have shown that vestibular stimulation affects sensory development in infant rats. The present study investigated effects of vestibular stimulation on 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 lesion, one group was rotated after lesions only, and a third group was never rotated. Developmentally, rotation significantly facilitated recovery of performance on the climbing over task, had a slight (but statistically significant) effect on the climb over task, and did not affect ability to balance on or traverse a beam. Following unilateral LVN lesions, rotation significantly facilitated recovery of performance only in those rats and only in rats which had been rotated since birth. Rats rotated since birth recovered righting reflex ability quickly following lesions. Rotation, either alone or before lesions, did not affect time spent on the balance beam. However, rats rotated since birth were more able to traverse the balance beam and less reacted to balancing maneuvers following LVN lesions. It seems that in both cat and man the adaptive process includes modifications of both behavior development and recovery of function following vestibular lesions, and that these modifications are dependent on the position of the cupula, the second group innervated a second region of the crista, and a third group innervated the broad end. Fibers that projected to the thin portion of the cupula also showed response characteristics that are dependent on the position of their associated receptors along the length of the crista. It is concluded that separate channels of vestibular input are preserved which are not available to the unadapted subject. Supported by Canadian MRC Grant No. MT-5630.


Exposure of the alert, initially unadapted, but vision-reversed cat to 4 hours forced oscillation in normal light produced (and highly significant) 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, for the same 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 significant adaptive change following 2 hours head rotation 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 reversal, i.e. VOR opposing eye movements during adaptation.

Supported by Canadian MRC Grants No. MT-5630 and MT-3557.
1954 TRANSFER FUNCTIONS OF CAT SEMICIRCULAR CANAL EIGHTH ORDER TRANSFER FUNCTION WITH A FRACTIONAL EXPONENT TERM.

In addition to linear neurons (CV greater than 10%) on the other hand, were best fit by a two pole, one zero transfer function similar in form to that observed for the same cells. Because others have noted a general decrease in gain as acceleration increases. A similar finding has been reported for Forelshuh muscle (GM) 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).


When labyrinthine motion is activated by sinusoidal horizontal rotation in decerebrate cats, vestibular reflex activation of neck and limb muscles lags 40-50° behind the discharge of sinusoidal horizontal afferents. Sensitivity of the anterior and posterior canal afferents is estimated to be approximately the same. A nearly linear gain relationship between that response and the input acceleration was obtained. The response of neurons, in and around the vestibular nuclei of anesthetized rats, to sinusoidal linear acceleration along the 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 Forelshuh muscle (GM) 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 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 Forelshuh muscle (GM) 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 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 Forelshuh muscle (GM) 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).


In contrast to the horizontal vestibulococcal reflex (HVOR), the vertical vestibulococcal reflex (VVOR) of the rabbit has a higher gain (eye velocity/head velocity) and a smaller phase lead (lag) in response to a horizontal canal stimulation. In the present paper, the gain and phase of the VVOR are similar to those of the HVOR. It is possible to derive a contribution of the unit to the VVOR by subtracting the VVOR obtained when the rabbit is "nose-up" from the VVOR obtained when the rabbit is "nose-down"; 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 unit 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 in the posterior canal signals. The magnitude 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 is 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 canal alone, although eliminating the VVOR, 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, impeded 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 relationship of these canals to the membranous duct which joins their ampullae to the utricle, thereby completing their endolymph "circuits." (Supported by PHS Grant EY-00848 and The Oregon Lions Sight Foundation).


Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

The Mauthner cell of the bullfrog tadpole (Bombina 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 produces short latency EPSP's and firing of the M-cell. The EPSP's 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 occurred after dorsal intracranial stimulation of the N-cell. Variability in the latency and amplitude of the tail response was observed with VIII nerve and intracranial stimulation. Intraocular stimulation at a frequency of 1 Hz evoked a tail response in the initial M-cell spike with no response to subsequent M-cell activity (Fig. B). The characteristic response to 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.)


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. Partially because of the ease with which compensation occurs, 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 gravastatic 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 7 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; b) hair cell, stereociliary or cuticular "circuit" distribution of the different forms of hair cell ciliary bundles; nor c) relative proportions of macular area covered by outward versus inward-oriented cuticular cells, which are not 1:1 but asymmetrical in the normal utricle.

We conclude that structural changes in the asymmetrically organized gravastatic 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.


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. Partially because of the ease with which compensation occurs, 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 gravastatic 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 gross asymmetrical disorientation. Animals were sacrificed at 7 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; b) hair cell, stereociliary or cuticular "circuit" distribution of the different forms of hair cell ciliary bundles; nor c) relative proportions of macular area covered by outward versus inward-oriented cuticular cells, which are not 1:1 but asymmetrical in the normal utricle.

We conclude that structural changes in the asymmetrically organized gravastatic 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.
1962 DYNAMIC EVALUATION OF HUMAN VESTIBULO-OCULAR FUNCTION USING WHITE NOISE ROTATION STIMULATION AND LINEAR SYSTEM PARAMETER ESTIMATION TECHNIQUES. Conrad Wall III, F. Owen Black and Dennis P. O'Leary. Dept. of Otologyngy, Div. of Vestibular Disorders, Univ. of Pittsburgh, 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. 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, 1973) to these experimentally derived gain and phase functions. Several different methods from small set of coefficients that can be directly related to mathematical models of the semicircular canal or of the VOR (Peterka, et al, 1973). 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.

In the second approach attempts to classify model 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.

References


Sinusoidal stimulation of single ampullary nerves with modulated continuous current 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.

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. 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 ones 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.

VISION
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 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 inhibited 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. Sturti). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115 and Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60687. 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 microprobes 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 200 KΩ 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 impaling pipette, showed both inward- and outward-going rectification. The amplitude of the light response could be altered by changing the membrane potential by 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.

The LP-pulvinar complex of the cat's thalamus contains a distinct mediodorsal zone, projecting input from the superficial layers of the superior colliculus (LPM), a second zone receiving input from the striate cortex (LP), and a third, lateral zone in the pulvinar (LPd). Receptive field properties of units in the thalamic projection layer were used to identify these three zones in normal material. In an attempt to define these zones anatomically, we injected the projecting areas with fluorescent histochemical techniques. The injections were centered in the striate cortex (LP) and the caudal lateral bank of the middle suprasylvian sulcus; the fundus and caudally, the lateral bank of the middle of the posterior suprasylvian sulcus and produced sparse labelling of the LPd in the anterior ectosylvian sulcus and the fundus of the lateral suprasylvian sulcus. The injection within the LPd labelled the ventral bank of the anterior ectosylvian sulcus; the fundus, caudally, the pulvinar of the superior colliculus; and those parts of the projection sulcus not labelled by LP injections.

This technique permitted the following: (1) The projection of thalamic neurons to distinct targets within the posterior association cortex. (2) The distinction of the LP-pulvinar complex of the cat's thalamus contains a dissection is possible with the locations of the center of the thalamic injection site. Injections centered in the precortical zone (LP) elicited heavy labelling in the cortex of the middle suprasylvian crown and in the fundus of the splenial sulcus, and variably labelled parts of the striate cortex. (3) The projection of thalamic neurons to distinct targets within the posterior association cortex. (4) The distinction of the LP-pulvinar complex of the cat's thalamus contains a dissection is possible with the locations of the center of the thalamic injection site.

Light activation of a cyclic nucleotide system that links 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 55 msec and t1/2 for the decrease is about 125 msec. Cyclic GMP levels are reduced by 10^5 to 10^6 molecules per photopigment molecule bleached at illumination levels below one photo pigment 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, that increases the level of cyclic GMP in ROS, can be decreased 70% (t1/2 = 7 sec) by low levels of illumination. ATP levels are not decreased.

Cyclic nucleotides have been shown to regulate cell physiology by stimulating the level of specific protein phosphorylations (protein kinase activity). Two small molecule weight proteins (MW, 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 H.B.).


The avian accessory optic nucleus, the nucleus of the bulbar optic root (nBOR), receives a specific projection from the displaced retinal ganglion cells. The nBOR has recently been reported to project bilaterally directly upon the oculomotor complex, trochlear and abducens 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 10^9-10^7 unit 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. Labeled fibers course caudally to terminate bilaterally upon the medial division of the inferior olivary complex (Olm) and the labeling was somewhat heavier over the ipsilateral Olm. Unilateral HRP injections into folia IXc, IXd or the paraflocculus of the vestibulocerebellum resulted in retrograde labeling of cells only within the contralateral Olm.

These results thus demonstrate the Olm which receives a bilateral nBOR projection, gives rise to a climbing fiber projection upon the olivary complex. The response facilitation is highly specific; 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 within the RF of the neurons and the response facilitation resulted in the monkey being able to make one of several responses: continue to fixate the spot and ignore the peripheral stimulus; make a saccade to a peripheral target; continue to fixate the spot, attend to the peripheral stimulus and release the lever 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 selective; 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 was 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 transient or sustained visual information processing.

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.

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 lateral 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 lobes (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, visual-spatial localization and food localization tasks. After pretraining, six bushbabies were given extensive striate lesions and were restested. 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 restested. A third group of animals with MT area lesions, which had been tested on similar tasks, received extensive striate cortex lesions and were restested (WT-17).

The results of these experiments were: (1) After extensive retraining, the final level of performance of five of the 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 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 the striate cortex and the amount of training the animals receive. (Supported by NIH grants EYO1444 and EYO0701)

1980 ULTRASTRUCTURAL EVIDENCE OF EARLY RETINAL GANGLION CELL DIFFERENTIATION IN XENOPUS LEAVIS. Charles Clémé and Philip Gross* (SPONS: D. P. Kimble). Dept. of Biology, University of Miami, Miami, Fl. 33136.

Ultrastructural evidence indicates that Xenopus retinal ganglion cell axons initiate 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 intercalaries of ventral retinal cells. Axons containing aligned microtubules and neurofilaments and elongated mitochondria with a paucity of other cell inclusions are found prior to Stage 32 possibly because they examined regions of the retinae 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 fascilitation. 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. Our evidence indicates that a significant number of ganglion cells become axon-bearing by Stage 28

(Supported by NIH grants EV01444 and EV00701)

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 existence 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 extend from the top to bottom, or from the top to the middle, of the visual space. 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 for these neurons as determined through each eye separately. The methods are not observed either in young kittens or in normally-reared adult cats, their existence provides evidence against the notion that atrophy of unstimulated cells underlies the effects of selected visual exposure.

Supported by Sigma Xi Grant in Aid to J.L.C. and O.N.R.
Contract # 900014-77-C-0173 to S.H.G.


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 a and b can be seen as accurate, 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 rotate, raise and lower an 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 90° 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 (mnemonic, for, or on the same hand) 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 always clear at the fixation point. This task was repeated 100 times. Mirror Index (MI) = m - ĭººalS

Ten subjects were found to have an MI greater than 20. Examination of the MI responses revealed that 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. The results show which responses under what conditions whether or not the hand crossed the midline of the body; left and right visual fields also elicited equal numbers of mirror errors implying that neither hemisphere is superior at this task. This work was partially supported by NIH Grant RR 07037.

To investigate the normal morphological parameters of retinal ganglion cells in the tree shrew, adult retinae were isolated, whole-mounted on slides, and fixed in glutaraldehyde. Following staining with methylene blue, the retinal ganglion cells were enumerated based on size and shape. The results showed a bimodal distribution of ganglion cells with a major peak at 25 µm² and a minor peak at 45 µm². There is a progressive increase in the average cell area as eccentricity from the central area increases (maximal at 150 µm² at 28º eccentricity to a mean of 63 µm² at 90º, and also an increase in the percentage of large cells (21%) at 13º eccentricity, 14% at 77º).

Injections of retinal ganglion cell axons in the area centralis revealed flat-vesicle and round-vesicle terminals on soma (7/100 µm²). 75% of the labeled cells contained reaction product, and 25% of the labeled cells were unlabelled. 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 K 007 EY 0061).


Cell classes in cortex have been defined on the basis of differences in morphology which are related to functional differences in connectivity. From an animal whose thalamocortical (lgn; 140 µm²) and thalamofugal (lgn) had been destroyed (4 day survival), we reconstructed these neurons from electron micrographs of 150 serial sections through layer IVa. 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-II border); apical and basilar dendrites; dense dendritic spines; flat-vesicle terminals on soma (13/100 µm²); lgn terminals on basal dendrites.

Class II: large stellate (20 µm²); dark cytoplasm; many flat-vesicle and round-vesicle terminals on soma (77/100 µm²; P<0.02); lgn terminals on cell body, primary, secondary, and tertiary dendrites.

Class III: stellate; varicose dendrites; gcl distribution of terminals on soma (13/100 µm²); lgn terminals completely restricted to secondary dendrites.

Class IV: radially elongated soma; apical dendrites shallower than radial, large spines; many flat-vesicle and round-vesicle terminals on soma (18/100 µm²); lgn terminals restricted to shafts of secondary dendrites.

Class V: small soma (74 µm²); dark cytoplasm; sparse distribution of flat-vesicle and round-vesicle terminals on soma (7/100 µm²; P<0.02); lgn terminals on soma.

Class VI: small soma (74 µm²); dark cytoplasm; sparse distribution of flat-vesicle and round-vesicle terminals on soma (7/100 µm²; P<0.02); lgn terminals on soma.

Class VII: pyramidal soma; apical dendrites sharply tapered; dendritic spines; gcl distribution of flat-vesicle and round-vesicle terminals on soma (190/100 µm²; P<0.02); lgn terminals on soma.

No gcl terminals were found on the spines or shafts of apical dendrites from deeper layers. We conclude that the results are different patterns to at least 6 classes of neurons in layer IVa and anticipate that the classes defined here by the differences in connectivity will show corresponding physiological differences. (WIN EY00828)


To investigate the normal morphological parameters of retinal ganglion cells in the tree shrew, adult retinae were isolated, whole-mounted on slides. Following staining with methylene blue, the retinal ganglion cells were enumerated based on size and shape. The results showed a bimodal distribution of ganglion cells with a major peak at 25 µm² and a minor peak at 45 µm². There is a progressive increase in the average cell area as eccentricity from the central area increases (maximal at 150 µm² at 28º eccentricity to a mean of 63 µm² at 90º).

Injections of retinal ganglion cell axons in the area centralis revealed flat-vesicle and round-vesicle terminals on soma (7/100 µm²). 75% of the labeled cells contained reaction product, and 25% of the labeled cells were unlabelled. 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 K 007 EY 0061).
1989 EFFECTS OF VISUAL DEPRIVATION ON THE DEVELOPMENTAL ANATOMY OF VISUAL CORTEX IN RATS. Timothy J. DeVoogd, James N. Cohn*, Michel Lichtenthaler*, Thomas R. Burnstine*, and William T. Greenough, Dept. Psychology and Neural and Behavioral Biology Program, University of Illinois at Urbana-Champaign, IL 61820. Researchers have shown that dark rearing results in reduced numbers of dendritic spines in visual cortex. These studies have generally not controlled either for the variance between groups 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 as follows. 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 on demand. The experiment ended when the rats were killed at 12, 15, 18, and 30 days of age. Their brains were removed, photographed, and stained with 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. However, 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 observed by Gyllensten and co-workers with dark rearing and may reflect differences in maternal cyclic activity allowed by the access to diurnal lighting in this study. Spine counts in striate and parastriate cortex are in progress. Supported by Grant NSF NS17-23660.

1990 CAT VISUAL CORTEX LACKS THE LUXOTONIC UNITS FOUND IN MONKEY. Edgar A. deLappe* 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. Int'l. Union Physiol. Sci. 13: 55, 1977. J. Neurophysiol. 37: 421, 1974). Unlike the “sustained” units identified in cat visual cortex, these “luxotonic” units do not require a light stimulus 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 (of “sustained”, not luxotonic) occur 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 results have been clear and consistent. 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 NINDS Grant NS30606.


If 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 spectrum in chiasm and tract appearing 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. 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. Int'l. Union Physiol. Sci. 13: 55, 1977. J. Neurophysiol. 37: 421, 1974). Unlike the “sustained” units identified in cat visual cortex, these “luxotonic” units do not require a light stimulus 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 (of “sustained”, not luxotonic) occur 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 results have been clear and consistent. 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 NINDS Grant NS30606.

1992 EXPERIMENTAL AMBLYOPIA - PRODUCTION BY RANDOM MONOCULAR SHUTTERS OF VISUAL INPUT. Frank H. Duffy, George D. Mower*, and James L. Burchfiel*. Seizure Unit and Neurophysiology Lab, Dept. of Neurology, Children's 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 dioptr 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.

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 cdm^-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^-4-10^-4 cdm^-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 DeVlois, 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, with the human, the spatial mosaic of the fly becomes functionally coarser as luminance drops. Possible physiological mechanisms underlying this behavior will be discussed.


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 section of the posterior corpus callosum at 15-19 days old (E), and an older surgical subject, at 29 days old (E29). Testing consisted of 10 - 2 minute trials per day starting at day 20 and continuing through day 40 old. For each trial, the subject was placed on a white and a black checked board 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, 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 crossed all responses to the deep side by 20 days old, and after 33 days old, descended from the centerboard on every trial. The E group chose the deep side more often and continued doing so through 39 days old (E29). The E group also refused to descend from the centerboard more often and continued doing so through 39 days old (E29). The 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 preference for the shallow side. In the N group, these two components develop simultaneously. Previous experiments have shown that cats in the E group have a tendency to have divergent visual fields and lose 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 interfering with early visual input so that depth cues that are present are ignored.


The frog's frontal organ is a photoreceptive system capable of discriminating between stimuli of different wavelengths. Previous physiological models have suggested that simple photo-receptor-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 axonophoresis of CoC12. A suction electrode filled with 10% CoCl2 solution was used to fill the frontal nerve axon, 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 arround areas of neuropil where some make ribbon-type synaptic contacts on each other. The pseudo-unipolar 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 CoC12 varied from 40-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C1240-105 which correlates well with our electron microscopic counts of the ganglion cells of the frontal organ are directly comparable to the number of cells labeled with C.
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 cell bodies, thick axons and dendrites, "radiate" dendritic branching pattern (DBP), narrow dendritic stratification in layer I, and large perikaryal field diameter (PDF). Class II cells have small cell bodies, thin axons, a "tufted" DBP with a profusion of dendritic appendages, broad PDF, and small PDGs. Class III cells have small-to-medium size cell bodies, relatively thin axons and dendrites, narrow PDF, and a wide range of PDGs 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 bistriate cell, and 2 kinds of "P-eptyn" cell (Lettvin et al., 1961). Dendritic field, cell body and axonal diameters of all but a few ganglion cells of classes II and IV increase with increasing distance from the visual streak. Most class I and class III cells can easily be assigned to 1 or 2 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 let or end order dendritic branches at tapering "initial segments". Slender beaded "axon collaterals" 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, some of which could 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. The retrograde 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 issue soaked in 10% atactic picric acid, 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 confine this observation. Retrograde transport was accomplished by lontophoretic application of a 4% solution of horseradish peroxidase (Sigma Type VI or Boehringer Grade I) dissolved in 0.5 M Tris-HCl buffer and of 0.2 M KC1 pH 8.6. Applications were made into either the dorsal raphe nucleus or the nucleus central superior nucleus. Grain counts were performed to confine this observation. The axons of the second type terminated primarily in layer IVc. Their axon diameters matched those of the small cells in laminae Cl-C3. They sometimes gave off collaterals to layer V or the lower half of the IPL. The third type of relay cell possessed large diameter axons (2.0-3.3µm, exclusive of myelin) while type 2 neurons had medium sized axons (1.0-1.7µm). Type 2 neurons gave off axon collaterals to layers II-V. In laminae I-C3, neurons with very fine axons (0.5-1.0µm), probably type 4 neurons, were found. Identification of type 1 and type 2 neurons was accomplished by the determination of their axon diameters, since these are appropriate for the conduction velocities reported for Y- and X-cells.)

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 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/m²) against a background of .086 cd/m². 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 made up the population. 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 cells 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.


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, 1976). 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 nerve 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 compression was 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 surgery to nerve III. The progressively appearing field compression, however, appeared 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.


Single and multi-unit recording from the pulvinar of 2 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 (VI=13%) and the dorsal areas (39%) were similar. 74% gave clear time-locked responses to visual stimuli and had properties similar to those reported in the geniculo-atriate 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


Both areas 17 and 18 receive input from the lateral geniculate nucleus. The extent to which these areas share the geniculate input 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 histochemical 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 200µ 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 areas 17 and 18 by axons that branch. In layers A and A1 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 ROI NS 06662 and ROI ET 00962.)

2006


There is a visual input to the rostro-medial pons which arises from layer V cells of the lateral geniculate nucleus. 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 in the same location. We next placed an array of stimulating electrodes in the superior colliculus. We recorded from cortical visual cells and tested 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=2.6 ms) were seen. 542 (N=11) of lateral suprasylvian corticopontine cells had bifurcated axons (pons latency=3.5 ms, colliculus latency=2.6 ms). The posterior middle-suprasylvian area (MSS) visual association area, has a heavy output to medial pons and colliculus (N=12) of MSS corticopontine cells bifurcated (MSS latency=5.5 ms, SC latency=4.4 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 cerebellum and the medial pontine nuclei, while the colliculus provides visual input to the vermis of the cerebellum via the dorsolateral pontine nuclei. The paraventricular 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. 195:105-129, 1955). Visuomotor information carried by the bifurcated axons is useful for visuomotor coordination of both proximal and distal muscles.

2007


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 have reported 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. Using sinusoidal grating stimuli similar to those employed by Hochstein and Shapley, we have found that concentrated neurons in the rabbit LGN can be classified into two non-overlapping groups depending upon whether they exhibit linear or nonlinear spatial summation. We have also found that properties such as size of receptive field center and type of response to standing contrast cannot be used to classify LGN cells as X or Y.

In contrast, 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


Visuobehavioral deprivation by monocular eyelid closure yields valuable insights into the development of the different visual pathways and into the relationship between collicular development of those pathways and the animal's capacity for visually guided behaviors. We have previously shown that the deprivation effects vary: they evoke axonal collateralization not only fiber outgrowth 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 from motor cortex in awake cats with implanted electrodes 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.

In 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 alone 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 responses in deprived cortex do not 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.

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 (Grueber, E.R. and Edin, S. E., J.C.N.179: 487, 1978) that the nucleus isthmus provides relay stations for the ipsilateral projection to frog tectum, and thereby provide one basis for binocular vision in Anuran amphibians. However, projections for nucleus isthmus to tectum may provide other functions besides binocular integration. Following large isthmus lesions, we often noticed two abnormal phenomena: 1) an increased 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.

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 Walker 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 extensions end before meeting. A second, narrow band of dense degeneration corresponds to the 18-19 border. Norstrally, 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. Norstrally, 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. 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 the interhemispheric projections is unclear, yet the pattern is reminiscent of that observed for the geniculostriate contribution to occipital dominance columns in the cat and monkey. This suggests that occipital dominance may be important in interhemispheric integrative mechanisms. (Supported by URC 8-1901-1311-39)


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. In contrast normal cats in which almost all visual projections 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 with the locations of efferent cells projecting to the dorsal lateral geniculate nucleus (dLGN) and the pulvinar (Pul). First, 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 expression 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 with the locations of efferent cells projecting to the dorsal lateral geniculate nucleus (dLGN) and the pulvinar (Pul). In the HH goggle condition, which produced the most dramatic effect, this ratio was 9. For the other conditions this ratio varied from 1.5 to 2.5. We conclude that while both goggle and drum rearing are effective, the combination of gogoles and asymmetrical orientations is the most effective way to alter the distribution of preferred orientations.

For electronmicroscopy, following enucleation and/or decentration (usually only of the striate cortex), the animals were allowed to survive from 2 days to several months. Differences between normal and decentered material in the projection area were apparent. The functional significance of the periodicity in the interhemispheric projections is unclear, yet the pattern is reminiscent of that observed for the geniculostriate contribution to occipital dominance columns in the cat and monkey. This suggests that occipital dominance may be important in interhemispheric integrative mechanisms. (Supported by URC 8-1901-1311-39)
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 amplitudes; 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. Conclusions: There are either separate nicotinic and muscarinic receptors in this retina, or there is a single type of receptor with mixed pharmacological properties.

Supported by NIH grant EYO0973 to L.M. Proenza.


Ten monkeys were deprived of binocular vision by suturing shut the lids of one eye (EYE 1) prior to the age of 24 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° 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 anesthesia, 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 multunit background activity was determined. In all animals the deprived eye drove some cortical units. NL cortex showed an ODC repeat in layer IV of 350μm/250/350μm, 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 transcranial autoradiography (TrAr) after H-proline & fucose injection into one eye which labeled dorsal lateral geniculate terminals in layer IV or Ca-deoxyglucagon (DG) tracing after reverse suture. The LGN (0.35-0.4) 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 1 to VI. The EL group after TrAr from EYE 1 showed clear dominance and orientation specificity of some single units and all multunit background activity was determined. In all animals the deprived eye drove some cortical units. NL cortex showed an ODC repeat in layer IV of 350μm/250/350μm, 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 transcranial autoradiography (TrAr) after H-proline & fucose injection into one eye which labeled dorsal lateral geniculate terminals in layer IV or Ca-deoxyglucagon (DG) tracing after reverse suture. The LGN (0.35-0.4) 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 1 to VI. The EL group after TrAr from EYE 1 showed clear dominance and orientation specificity of some single units and all multunit background activity was determined. In all animals the deprived eye drove some cortical units. NL cortex showed an ODC repeat in layer IV of 350μm/250/350μm, 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 transcranial autoradiography (TrAr) after H-proline & fucose injection into one eye which labeled dorsal lateral geniculate terminals in layer IV or Ca-deoxyglucagon (DG) tracing after reverse suture. The LGN (0.35-0.4) 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 1 to VI. The EL group after TrAr from EYE 1 showed clear dominance and orientation specificity of some single units and all multunit background activity was determined. In all animals the deprived eye drove some cortical units. NL cortex showed an ODC repeat in layer IV of 350μm/250/350μm, 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 transcranial autoradiography (TrAr) after H-proline & fucose injection into one eye which labeled dorsal lateral geniculate terminals in layer IV or Ca-deoxyglucagon (DG) tracing after reverse suture. The LGN (0.35-0.4) 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 1 to VI. The EL group after TrAr from EYE 1 showed clear dominance and orientation specificity of some single units and all multunit background activity was determined. In all animals the deprived eye drove some cortical units. NL cortex showed an ODC repeat in layer IV of 350μm/250/350μm, 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.

While the superior colliculus is known to project to a variety of structures in the brain, it is not known how the laminar organization of these structures is determined. Our experiments were designed to determine the distribution of the cells which project to each of these structures. 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 or five pathways from the superior colliculus: the predorsal bundle and the ipsilateral tektocobular pathway. After survival times ranging from 24 to 48 hours, the brains were fixed by aldehyde perfusion. Analysis of the labeled cells was according to modified LaVail and LaVail (DAB) and/or Mesulam (BDHC) protocols.

Following single 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 and only a few scattered labeled cells (83-88%) were located in stratum griseum superficiale. Distribution of these cells by labeling them with retrogradely transported horseradish peroxidase (HRP).

We conclude from these experiments that the majority of axons in the predorsal bundle arise from cells located in stratum griseum intermediare of the contralateral superior colliculus, whereas the majority of axons in the ipsilateral tektocobular pathway arise in the stratum griseum profundum. The results not only provide striking confirmation of an old observation concerning the horizontal spread of the parvocellular layers, but also demonstrate that there is a specific projection to the lateral geniculate nucleus of primates.

2018 DISTRIBUTION OF INPUTS FROM THE TWO EYES TO STRIATE CORTEX OF SQUIRREL MONKEYS D. H. Rubel 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 binocular and monocular eye stripes. A similar segregation has recently been reported in the squirrel monkey (Florence and Casagrande, ARVO Abstracts, Inv. Ophthal. 4, Vis. Sci. suppl. Apr. 1974, p. 291).

Squirrels, following our technique of tritiated amino acid into the eye, the transneuronally labeled cells as in autoradiographic distribution is not in discrete stripes along IV c but uniformly (Rubel, Wiesel and LeVay 1976 Cold Spring Harpr 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 variations in density of label, especially in the upper part of layer IV c (IV c c). Opposite the more densely labelled regions we found faint aggregations of label situated above the upper tier of layer IV a, occupying much of layer III.

In the squirrel monkey the lateral geniculate layers are less clearly defined than in the macaque. There is thus a greater risk that labeling may diffuse from the injected eye to the other layers; this could tend to produce false positive results. In the squirrel monkey there is a well defined ocular dominance first slab (s) of layer IV c, and the cells driven about equally from the two eyes (just as in the macaque) are in the most superficial portion of IV c. In the squirrel monkey there were few labeled cells in the middle layer of IV c. There were also fewer labeled cells in the upper layers; there was a period of fluctuation in dominance from one eye to the other at intervals of about 250 µm. The squirrel monkey therefore may have a homogenous layered cortex, though they are probably narrower and certainly less well defined than those of the adult macaque.


 Autoradiographic tracing procedures have been used to study the organization of retinogeniculate axons in seven primate species. 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 retinogeniculate layers is preserved in the prosimians, one species of Old World monkeys and two species of prosimians. These data suggest that the basic primate pattern of retinogeniculate layers is preserved in the prosimians, one species of Old World monkeys and two species of prosimians. These data suggest that the basic pattern of retinogeniculate organization is six layers, but not the traditional six. Prosimians have evolved two additional layers, the koniocellular layers are in the lower layers. In the upper layers, there is a uniform layer IV c, and a distinct layer IV c a. In the Old World monkeys, the koniocellular layer IV c a is composed of layer IV c, and a distinct layer IV c a. In the New World monkeys, the koniocellular layer IV c a is composed of layer IV c, and a distinct layer IV c a. In the New World monkeys, the koniocellular layer IV c a is composed of layer IV c, and a distinct layer IV c a. In the New World monkeys, the koniocellular layer IV c a is composed of layer IV c, and a distinct layer IV c a. In the New World monkeys, the koniocellular layer IV c a is composed of layer IV c, and a distinct layer IV c a. In the New World monkeys, the koniocellular layer IV c a is composed of layer IV c, and a distinct layer IV c a.
2021 VISUAL FIELD DEFECTS AND MORPHOLOGICAL CHANGES RESULTING FROM MONOCULAR DEPRIVATION IN PROSIMIAN PRIMATES. Bhawna 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 either anatomical and/or behavioral subjects. We examined the visual field of 8 monocularly deprived and 2 normally reared subjects using a penny test technique previously described (Sherman, Brain Res. 49: 29, 1973). When tested with the left (non-deprived) eye, all subjects exhibited 135° of vision, i.e., from 90° into the ipsilateral hemifield and 45° into the contralateral hemifield. When tested with the right (deprived) eye alone, the deprived subjects responded initially as if blind. After 1 week, the deprived eye began to respond to stimuli presented from 45° to 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 equally.

After testing was completed, 4 of the deprived behavioral galagos and 3 normally reared animals received an eye injection of 3H 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. Neurol. 114: 48, 1958). In the 3H proline 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 labeling 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 in a given projection, 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)

2022 PROJECTION OF THE RETINA TO THE LATERAL GENICULATE NUCLEUS IN THE CAT FOLLOWING NEONATALABLATION OF VISUAL CORTEX. R. E. Kalil

- Department. Anat., Univ. Miss., 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 AI, and they are especially prominent ventral to AI in the region of the C laminae.

Retrograde degeneration is not confined to the LGN, but also occurs transynaptically in the retina. Thus in the ipsilateral temporal and contralateral nasal hemiportio 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, Piek-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 labeled proline. Following survival periods of 7 to 14 days, the animals were perfused with 10% formal-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 common, 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 network 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 labeling indicating a nonuniform pattern of innervation.


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, the first small spot response following the large spot sequence is greater than the preceding small spot response. TRE is strong in the proximal negative response and N-wave, the proximal K+ increase, and larger 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.

Physiologically, 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 Wehrbin's retinalin effect, may arise from interactions in the proximal retina.

Supported by NIH Grant EY-00973 to LMP.

2023 TRANSIENT RESPONSE ENHANCEMENT IN MUDPUPPY RETINA. Chester J. Karwoski and Luis M. Proenza. Department of Psychology, Univ. Georgia, Athens, GA 30602


The results presented here suggest that this phenomenon may have a component of retinal origin and that, like Wehrbin's retinalin effect, 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 Karwoski, Chester J. Karwoski, and Luis M. Proenza.

Three regions of the bushbaby's visual cortex receive nonoverlapping projections from three thalamic areas 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 and superior pulvinar-extrastriate systems function in different visual tasks and resemble higher mammals' 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 three normal control and three operated groups having bilateral cortical lesions in area 17 (G), MT, or areas 18 and 19 (X). 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 discrimination tasks 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 X5 was also impaired in these tasks but showed less severe deficits in visual learning and sensory capacity than Group MT.

These results suggest that each of the three thalamic-cortical 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: MYOPE OR NOT? Albert H. Kirsch 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 rabbit monkey resulted in fewer terminals in the central sutured eye. Among those was elongation of up to 21% with resulting axial myopia (-1.5 diopters). Gollender et al. (1976) reported that in the cat the eyes develop normally following lid suture with no axial elongation.

If 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 was often hyperopic. While the kitten study was in progress, we obtained a 4 month old thesauus monkey and immediately sutured one eye closed. Since our data was apparently differing from earlier reports, we wanted to use the monkey as a control and see if it also differed. We followed the growth of the eyes on kittens whose eyes were sutured before or just entering the critical period. The scan ultra-sonography revealed a difference in axial length between the sutured and normal eye on each animal with the sutured eye was often hyperopic.


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. 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 neurones with their dendritic fields extended eccentrically about the cell body while the neurones of the deep division are predominantly stellate cells.

The deep division may be partitioned into at least three subdivisions. The superficial subdivision (0–25 µ below the surface) has marginal cells in its zonal portion, horizontal cells in its central portion which branch out extensively. The superficial subdivision contains a mixture of narrow field vertical cells and stellate cells with occasional wide field vertical cells.

We have studied these relationships by placing a photocell near a cell's receptive field and by recording unit responses in relation to receptive field location. We have used these relationships by placing a counterphase grating with the same phase relation as that used by Wiesel & Raviola (1977) to test the hypothesis that most simple cells receive input predominantly from one or off-centre LGN neurones. This is consistent with 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. The deep division has 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. The deep division has 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.

2027 PHASE RELATIONSHIPS OF RESPONSES TO MOVING SINUSOIDAL GRATINGS OF SIMPLE CELLS IN CAT STRIATE CORTEX. B.B. Lee, A.Elephant, and V.Viru. Max Planck Institute for Biophysical Chemistry, D-3400 Goettingen, F.R.G. 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. In the present study, we determined the critical flicker frequency of single cell responses in relation to receptive field location. We have studied these relationships by placing a counterphase grating with the same phase relation as that used by Wiesel & Raviola (1977) to test the hypothesis that most simple cells receive input predominantly from one or off-centre LGN neurones.
2029 BEHAVIORAL DEMONSTRATION OF MCCOLLUGH 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 stimulus.

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 and move one eye under alternating vertical and horizontal gratings of complementary color. Following adaptation a test grating whose color changed from red to green or green to red was presented. Monkeys were trained to release a response lever during the interval that the grating was white. After adaptation, there were orientation specific changes in the monkey's visual system. As predicted if the animals were experiencing a McCollough effect. This work was supported by NIH grants 5 T32 EY 07019-03 and 1 RO1 EY 02230-01 and NIH grant 5 S07 NR 05400-16.


In this study we have made use of a technique of selective inactivation of localized regions of brain tissue by means of microinjections of the local anesthetic lidocaine. Injections were made through a micropipette onto which had been placed 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 of single units in diameter of 3-10 mm in 3 to 8 min. 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 the lower lateral geniculate layer (L) cortex. Previous work has shown that parvocellular layers (5-6) are comprised mainly of color-opponent cells, while the magnocellular layers (1 and 2) contain inactivates the effects of adaptation. 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 parietal region of the monkey striate cortex. Control experiments demonstrated that the injections were restricted to the laminar level and did not interrupt retinal or geniculo-cortical fibers for the retinotopically corresponding parts of other laminae. The results are as follows:


2. Some complex cells are driven exclusively by either parvocellular or magnocellular input, while others receive excitatory input only in 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 BNS76-8254, NIH grant R01 EY00676 and NS05477-01A1, and NINCDS grant 5 P01 12356-03.)


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 reversal frequency) were independently varied. The amplitudes were generated on an oscilloscope and had a mean luminance of 33 cd/m2. The CSFs represent contrast threshold for evoked neuronal discharge as a function of spatial and 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. One other difference between X- and Y-cells was evident if 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- and Y-cells with activity in visual cortex will be sensitive to higher spatial frequencies and less sensitive to higher temporal frequencies. However, considerate overlap between 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 events. While observations of single neurons have provided evidence that such effort is difficult to reconcile, for example, with the neural pattern of activation evoked by an oriented visual target in Area 17 by the mechanism of the 2-dimensional code of localization (Hubel, Wiesel and Stryker, J. Comp. Neurol., 177, 361-380, 1978) or by single unit recording. On the other hand, by utilizing the population response pattern 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. In contrast grating targets under computer control were superimposed upon otherwise stabilized retinal images to produce adaptation. Our neurophysiological results suggest that 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 orientation adaptation is 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 effector neurons that form the initial segment of the cortical pathway subserving pattern discrimination. Reconstitution of the activation pattern of the effector neurons was accomplished by means of a mathematical model incorporating the distribution of orientation information and with the function describing the decrement induced by adaptation. Orientation channels, defined by the elevation in threshold produced by adapting displays containing 100% 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.)

At least 12 classes of ganglion cells, 12 classes of amacrine cells, and 5 classes of bipolar cells 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 cells in a small patch (50 µm x 50 µm) of cat retina reconstructed from electron micrographs of 150 serial sections. At least 12 classes of ganglion cells, 12 classes of amacrine cells, and 6 classes of bipolars can be distinguished in mammalian retina, according to Cajal, based on differences in cell volume, dendritic morphology and pattern of synaptic contacts.

- Large soma (3 x 10^3 µm^3); several thick primary dendrites to inner 1/3 of inner plexiform layer (IPL) (near the ganglion cells); proximal dendrite to middle 1/3 of inner plexiform layer (IPL) (near the ganglion cells); distal branches to outer 1/3 of IPL; distal branches receive contacts (2 cells).
- Medium soma (1 x 10^3 µm^3); one or more dendrites to middle 1/3 of IPL; distal branches receive contacts (2 cells).
- Small soma (1 x 10^3 µm^3); single fine dendrite to inner 1/3 of IPL; proximal dendrite receive 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 unsymmetrically. Four classes of amacrine cells 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 subclasses 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 EY008286)

AUTORADIOGRAPHIC LOCALIZATION OF ACETYLCOLINE 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 acetylcholine into acetylcholine (ACh), and to distinguish them from sites where choline serves primarily as a lipid precursor. All retinas were incubated in vitro for 30 min in a medium containing 0.3 mM 3H-choline. Some were then incubated for a subsequent 1 h under constant release of 3H-ACh and 3H-choline (flushing light, 1 mM unlabeled choline). Others were incubated under ACh-protecting conditions (20 mM Mg++, 0.1 mM unlabeled choline). Each of 4 levels of retina was analyzed chemically. A small piece was fixed with osmium tetroxide. The specimen was then washed and processed for autoradiography. The radioactivity responsible for these grains was found to be distributed almost exclusively in the inner plexiform layer (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 retinal ganglion cells. Autoradiography at these sites provides invaluable information about the association of acetylcholine and the photoreceptor outer segments. 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. The radioactivity responsible for these grains represented ACh that was released from degenerating photoreceptors that were exposed to water or from tissue initially fixed with aqueous osmium tetroxide. It was further confirmed by direct chemical analysis of the photoreceptor outer segments present 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. 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 amacrine cells. 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.


The bulk of the cat’s retino-collicular projection crossed in the monkey and the stratum zonale. This region, extracellular microelectrodes recorded 1-2 mV negative waves in response to stimuli faster than 15º/s. The results of these experiments were similar to those of previous studies on the relationship between JZPs and cellular action potentials recorded concurrently, but optic tract stimulation produced a rapid but reversible decline in JZP amplitude. The relationship between JZPs and cellular action potentials was highly reordered 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 recruiting 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 and reversible decline in JZP amplitude. The relationship between JZPs and cellular action potentials recurred 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 summed JZPs. Extracellular currents, resulting from the subthreshold activation of multiple and disynaptic JZPs, caused the JZP. (Supported by PHS NS 09977 and BY 025050)
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 following tectal ablation have sustaining connections in non-tectal areas. To test this hypothesis, the eyes contralateral to tectal lesions were studied in retinal whole mounts and in material sectioned for light and electron microscopy. At 15 days of incubation, these cells showed projections to the non-tectal nuclei comparable to those of the ganglion cell layer following tectal ablation have sustaining connections in non-tectal areas. Several characteristics of these cells showed changes in the ganglion cell mosaic. 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 eectomammillary nucleus. These results demonstrated that at least some of these 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-EYO1477 and the Helen Regenstein Fellowship.


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 calcineurin system 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 limit of the temporal MTF was shifted about 1/2 octave toward lower frequencies. Low luminance sensitivity was 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 constriction of the visual field seen in perimetry is one index of peripheral impairment. Loss of peripheral vision should also degrade sensitivity to high luminance-highest 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 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.


PSP analysis of retinal ganglion cells in the goldfish was carried out in the perfused retinal eyecup preparation of rabbit and mudpuppy. Intracellular current injection, conductance measurements and the selective blockers of GABA receptors were used to examine the synaptic input to the ganglion cell. Excitatory, inhibitory, and dysfacilitatory PSP components were observed in each cell type. Type I on cells receive a sustained inhibitory input after an initial EPSP: Type I off cells receive a phasic PSP at light off. These responses reflect a strong inhibitory input from a subtype of amacrine cell. Some evidence suggests that the sustained inhibition of on cells is GABA mediated, while that for off cells is Glycnergic. Type II and IV cells receive little or no amacrine cells and show prominent PSPs at high and off PSPs. When the EPSPs are fully blocked with bicuculline or strychnine, both on and off EPSPs are observed in both types of data suggesting that they receive a sustained inhibitory input from both bipolar cell types, but under normal conditions, one of the EPSPs is obliterated by the powerful inhibitory action. Off-on ganglion cells receive excitation from both bipolar cell types and are strongly inhibited by on-off amacrine cells. These on-off EPSPs are blocked by either bicuculline or strychnine. Very few cells show a selective block of the off EPSP with strychnine, suggesting that a small proportion of on-off cells receive inhibition from separate receptor sites.

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 off ganglion cells receive inhibition, but other ganglion cell types receive inhibition from amacrine cells according to several somewhat stereotyped patterns.

Supported by NIH Grants EYO0884 and EYO1802.
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); visual stimuli restricted to central visual field only (C); vision restricted only to peripheral visual field (500° PO). The behavioral response was described in terms of Fourier coefficients for longitudinal position at the forced frequencies of the platform and visual field movement. 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 movement during C, PO and N. Movement of the platform and peripheral visual scene during H provided conflicting visual input from the oscillated peripheral and stationary central visual scenes. Hence all 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 visual field inputs reduces peripheral field influences on postural control. These data indicate that postural control processes are subject to influences from both central and peripheral visual fields.

Postoperatively, the response to platform oscillation alone during H and B was unchanged. The differences between responses in B and conditions persisted in the absence of the visual cortex. Peripheral visual field movement induced a similar response during either B or PO, and no postural response could be induced by visual field movement during C. 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)

A SPATIAL FOURIER ANALYZER BY ANY OTHER NAME IS AN EDGE DETECTOR.

Models of pattern vision which postulate some form of spatial Fourier analysis of visually evoked responses have recently received considerable attention. Moreover, both spatial frequency response and line weighting data can be used to make accurate predictions of responses to broad bars and to complex gratings. 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 profiles and sinusoidal grating stimuli. 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 can 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 to use the results 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.

Support by NSF (BNS 76-18904) and NIH (EY 1977).

RAPID RECOVERY FROM VISUAL DEPRIVATION IN NEURONS OF CAT PARASIGRISTATE CORTEX. Michael J. Mustari and Max Cyander.
Dahousie 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 experimentally. These cells were studied during adaptation to the visible world and were also tested with methods for stimulus presentation and data collection. Particular attention was directed towards orientation and direction selectivity of the firing of single neurons. A neuron was considered orientation biased (OB) or direction selective (DS) if the ratio between number of spikes in the best orientation or opposite direction was 0.5 or less. Some OB cells were classified as orientation selective (OS) if they failed to respond to either orientation. The results indicate that cortex functions reduce center-surround inhibitory processes within the lateral geniculate nucleus.
2045 BLOODINESS IN MONKEYS AFTER LESIONS OF NONVISUAL CORTEX: NOT A VISUAL-MOTOR DISCONNECTION EFFECT. Richard K. Nakanura 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 behavior that deviated from the normal visual behavior (Neuroscience, 3:571, 1977). The lesion, placed in one hemisphere, completely spared both the visual cortex (striate, prestriate, and inferior temporal) and the motor cortex (motor, premotor, ventral premotor, and cingulate) but included all other cortical areas: the other hemisphere was visually deafferented by optic tract section and frontal commissurotomy. Under general anesthesia, it was carefully deafferented such preparations indicated that, in the hemisphere with the ablation, the geniculostriate path was intact as intended. Nevertheless, the animals were behaviorally ablated for 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 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 connect the striate to prestriate border) to the motor system. This analysis implies that the territory ablated produced blindness are known to produce neglect. Accordingly, the territory ablated and the forebrain commissures normally provide some activating input, direct or indirect, that is necessary for visual processing input to the visual system. 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 second, larger lesion in another monkey's hemisphere did not 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. Other localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a non-specific 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 cor- tex. This is a new area of research requiring a more complete understanding of the mechanisms underlying visual processing, though a less complete loss than that producing blindness. Supported by NIMH fellowship F132-MH0273.


In recent years, it has become clear that microcircuitry of the retina is of considerable help to label neurons by means that may relate their morphology to their function. We injected eyes with radioactive transmitter, GABA or glycine (100 μCi in 10 μl.) and perfused the animals one hour later. Series of 70-200 consecutive sections were processed. The retinal central was prepared as electron microscope autoradiograms. In the GABA experiments 2% of the cells in the amacrine layer were labeled for 100 μCi (3X background) and proved to be "interplexiform" cells (ICPs) whose reconstructed processes could be followed into the retinal inner plexiform layer (IPL), where they made conventional synapses. There were about 90 ICPs/mm², but they were unevenly spread, being sparse in some areas (80-95 μm apart) and absent in others (elliptical regions averaging 320x160 μm). An additional 20% of the amacrine layer cells were also labeled but less heavily (9X background). These were true amacrine, slightly larger and darker than the ICPs, with processes reconstructed through the outer 1/3 of the IPL. That GABA-labeled amacrine processes were also found diffusely through the IPL, particularly in the innermost regions (ganglion cells) suggests that the GABA amacrine are of a diffuse or multi-stratified variety. In the glycine experiment, 15% of the amacrine layer cells were heavily labeled and sent processes radially through at least the outer 1/3 of the IPL. An additional 3% of the amacrine layer cells were labeled, but more sparsely. These sent one or more stout processes vertically through at least 2/3 the depth of the IPL. Large (2-3 μm) varicosities were seen at all levels of the cell bodies and the vertical processes and were also labeled. We conclude that amacrine 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 EIO0828).


The origin of the electroretinogram b-wave response was investigated by determining its current source density distribution in the dark adapted eye 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 directing incremental tissue resistance. Current source density was calculated from the spatial derivative of the current density. These measurements show that the b-wave is generated by a proximal current source which is restricted to a narrow (∼10μm) 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 distal portion of b-wave current 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 Hiller (Neuroscience Abstracts, 1977). The currents associated with these 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. This observation provides strong support for the model of b-wave generation based on passive Müller cell depolarization driven by increases in [K+]o. (Supported by NIH grant EY00094 and a grant from the Bell Telephone Laboratories, Inc.)

2048 DESCRIPTIVE AND QUANTITATIVE EM STUDIES OF THE OPTIC TECTUM OF XENOPUS FOLLOWING ENUCLEATION. J. Jorden, A.-J. C. Ortegø 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 new fibers, which occur in the superficial tectum, occurs after deafferentation. By 5 days, the total number of synapses on the denervated side drops to 50% of the control side. This decrease in the number of synapses is still apparent 4 mos. following enucleation. Few 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. In our study, all the sprouted to occupy post-synaptic sites vacated by degenerating optic nerve terminals. In order to compare this, the two studies, the authors of the present study counted in the superficial 800 synapses in normal and denervated tecta in Xenopus were characterized according to a number of parameters that are often paid to retinal morphology and type of synaptic contact. In Xenopus, however, the type of synapse was found in the number of flattened and spherical vesicle containing profiles making asymmetric contacts. It 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 due to gliosis, these new synapses remain uninnervated. These findings have important implications for how synaptogenesis must occur during retinal development and for synaptic sites remain uninnervated. This hypothesis is consistent with the following findings. First, the number of flattened and spherical vesicle containing profiles making asymmetric contacts is reduced by 80% following enucleation. Second, the number of flattened and spherical vesicle containing profiles making asymmetric contacts is reduced by 80% following enucleation. Third, the number of flattened and spherical vesicle containing profiles making asymmetric contacts is reduced by 80% following enucleation.

Cells by two-dimensional 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.


Cats were reared from birth to at least one year in a room illuminated by a short duration strobe flash (40/400). This illumination provided pattern visual stimulation but precluded the perception of motion. Earlier we reported (Pasternak, Merigan and Brown, Society for Neuroscience Annual Meeting, Göttingen-Nikolausberg, Nest-Germany) 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 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 6-50 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.

STROKE 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) to eye movements and 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 had not trained to make eye movements (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 movement (e.g., the monkeys decreased to 50% of maximum as the direction changed by 30° - 60°).

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 the 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 maximally active only if the stimulus was not 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 displacements 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.

In Dipsosaurus dorsalis, the optic tectum consists of: 1) the superficial strata, composed of seven alternating cell and fiber layers; 2) the mid-dorsal tectum, and 3) the ventral rim, each of three monolayers of somata alternating with cell free zones. In addition to this pattern of lamination, the tectum in Dipso- saurus exhibits areas of regional specialization. The mid-dorsal tectum, the lateral tectum, and the ventral rim are areas of 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 mid-dorsal tectum 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 tec- tum can be distinguished by the presence of all survival times, of two bands of large caliber fibers which enter the tectal layers parallel to the surface and run approxi­ mately 1/3 the way into the tectum. These are accompanied by coarse arborescical dendrites, particularly over the deeper fiber layer. The three dense bands of silver particles associated with the lateral tectum do not extend to 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 regions of Dipsosaurus topographically onto the tectal surface, these subdivisions of the tectum may reflect regional specializations in the ganglion cell layer of the optic tectum. (Supported by A.P. Sloan Postdoctoral Fellowship and PHS Grant NS 12518)

VISSON

INVESTIGATION OF SECONDARY COMMISSURES IN INTERHIMERIC TRANSFER M. Pitcairn, G. Page, F. Lapierre and M. Blaszczyk. Lab. de Neuro­ psychologie, Univ. du Quebec, Trois-Rivieres and Univ. de Montre­ al, Montreal, Canada.

Previous studies have shown that interhemispheric transfer is present in animals bearing a transection of the corpus callosum and the optic chiasm. Such transfer only in the case postulated for visual information seems to be mediated via the midline secondary of subcommissural commissures. The present experiment was undertaken to evaluate this hypothesis. Adult cats underwent a section of a left optic tract (LOT) and a constriction of the midline commissure (MC) to the visual- lateral hemisphere (17, 18, 19 and suprasyl­vian area). The intact direct deafferented hemisphere is thus only innervated by the physiological defined X and Y cells have been associated with the RT and F synapses received by any particular D element. Indivi­ dual D processes which are part of triads in the high triad involve are relatively smooth, but show small appendages and translucent cytoplasm, but one (F profiles) contains loosely packed pleomorphic vesicles and no ribosomes. The second type (D profile) exhibits numerous ribosomal cosettes, but no vesicles. Triads containing only RT synapses and only F synapses have the same or greater effect on ganglion cell threshold. This suggests that a triad in the low triad group of RTs is less involved than those found on the dendrites of Guillery Class II cells. The presence of background light increases sensitivity of the receptor potential to threshold potential for the receptor potential and the b-wave) and the intensity-response function (threshold of spikes above manipulated 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 10 times greater for the receptor potential than for either the b-wave or the ganglion cell. However, the intensity-response functions of all three measures were similar in shape when response was expressed as percent of maxi­ mum. Backgrounds that decreased b-wave threshold by 1 0 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 cell's. The presence of background lights increased the b-wave threshold of ganglion cell but the 50-fold had no effect on receptor potential threshold or intensity-response function. We have shown that the receptor potential, ganglion cell in the rat is similarly affected by the presence of background lights, but differently in their presence. Our results are in good agreement with those of Green et al. and with earlier work by Wolf and' in the cat. We conclude that the light adaptation in the rat resembles those in the skate, and we suggest that they may be similar in other vertebrates as well. (Supported by EY00379 to D. G. S.)


INVESTIGATION OF SECONDARY COMMISSURES IN INTERHIMERIC TRANSFER M. Pitcairn, G. Page, F. Lapierre and M. Blaszczyk. Lab. de Neuro­ psychologie, Univ. du Quebec, Trois-Rivieres and Univ. de Montreal, Montreal, Canada.

Previous studies have shown that interhemispheric transfer is present in animals bearing a transection of the corpus callosum and the optic chiasm. Such transfer only in the case postulated for visual information seems to be mediated via the midline secondary of subcommissural commissures. The present experiment was undertaken to evaluate this hypothesis. Adult cats underwent a section of a left optic tract (LOT) and a constriction of the midline commissure (MC) to the visual- lateral hemisphere (17, 18, 19 and suprasyl­vian area). The intact direct deafferented hemisphere is thus only innervated by the physiological defined X and Y cells have been associated with the RT and F synapses received by any particular D element. Indivi­ dual D processes which are part of triads in the high triad involve are relatively smooth, but show small appendages and translucent cytoplasm, but one (F profiles) contains loosely packed pleomorphic vesicles and no ribosomes. The second type (D profile) exhibits numerous ribosomal cosettes, but no vesicles. Triads containing only RT synapses and only F synapses have the same or greater effect on ganglion cell threshold. This suggests that a triad in the low triad group of RTs is less involved than those found on the dendrites of Guillery Class II cells. The presence of background light increases sensitivity of the receptor potential to threshold potential for the receptor potential and the b-wave) and the intensity-response function (threshold of spikes above manipulated 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 10 times greater for the receptor potential than for either the b-wave or the ganglion cell. However, the intensity-response functions of all three measures were similar in shape when response was expressed as percent of maxi­ mum. Backgrounds that decreased b-wave threshold by 1 0 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 cell's. The presence of background lights increased the b-wave threshold of ganglion cell but the 50-fold had no effect on receptor potential threshold or intensity-response function. We have shown that the receptor potential, ganglion cell in the rat is similarly affected by the presence of background lights, but differently in their presence. Our results are in good agreement with those of Green et al. and with earlier work by Wolf and' in the cat. We conclude that the light adaptation in the rat resembles those in the skate, and we suggest that they may be similar in other vertebrates as well. (Supported by EY00379 to D. G. S.)


STRONG AND WEAK TRIADIC INPUT TO RELAY CELLS OF THE LATERAL GENICULATE BODY AND THEIR INNERVATION BY THE LATEX BROWN RABBIT. C. Badaracco and J. H. LaVail. Dept. of Anatomy, Howard University, Washington, D. C. 20059

Nineteen terminal boutons of retinal fibers in the dorsal lateral geniculate body have been examined throughout their extent in long series of uninterrupted, consecutive thin sections. The profiles postsynaptic to the retinal terminal 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 cosettes, but no vesicles. Triadic arrangements of one or two (D) and one (F) profiles postsynaptic to an RT is presynaptic to a D profile which is presynaptic to one 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 received about equal number of RT and 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 smooth, but show small appendages and resemble the dendrites of Guillery Class I cells. The hypothesis that the function of the triad is a feed­forward inhibition was tested by injecting aspartate into the vitreous (supported by Grant #NS-11614) and Davis' finding that the F profiles accumulate DABA. Since the physiologically defined X and Y cells have been associated with Class I and II respectively, one would predict that the RT and F synapses taken by injecting aspartate into the vitreous (supported by Grant #NS-11614) and Davis' finding that the F profiles accumulate DABA. Since the physiologically defined X and Y cells have been associated with Class I and II respectively, one would predict that the RT and F synapses
In birds, the tectum gives rise to two major descending systems, the ipsilateral tectopontine-tectoreticular pathway (TPP and the contraterminal tectobulbar component (CTB). The CTB innervates within the lateral reticular formation (FLR) of the brainstem and within the lateral pontine nucleus (LP). The CTB decussates at the ventral tectoammonial commissure (VTC) and terminates in the paramedian brainstem. These two major descending tectofugal pathways have been described in all vertebrates studied. The horseradish peroxidase technique used in the present study was to determine the laminar distribution of the cells of origin of these descending tectal systems in the pigeon.

In the chicken, injections into the region immediately caudal to the field of the CTB, at a variety of caudal pontine and rostral medullary sites resulted in labeled cells exclusively in layer 13 of the contralateral dorsal tectal, 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. 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 central and terminal portions of the CTB arise from 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. If particular care was taken to ensure 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 non-reciprocal layers and have small visual receptive fields (1-10 degrees).

Supported by USPHS grant I P32 HS 05682-01 to A.R.


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 caudal pole of the thalamus. The connectional organization of this region was studied using retrograde (HRP) and anterograde (sodium-Hexahydroxydiaminopropionic acid) transport methods. Injections of HRP into layer 1 of the lateral pulvinar resulted in labeled cells in the ipsilateral temporal cortex (areas 18 and 19) and revealed a highly convergent input to PLγ. Interestingly, discrete microinjections (0.1 µl) of HRP and amphibian acid revealed reciprocal projections to perirhinal cortex. Instead WPL was found to project to the ipsilateral temporal lobe. More specifically, WPL has a cortical target that includes layers 1, 2, 3, and 4 of inferotemporal cortex (areas 20 and 21). This projection to cortex follows a loose topographic organization such that ventral regions of PLγ project to anterior portions of inferotemporal cortex while dorsal PLγ projects to more anterior and ventral regions of inferotemporal cortex. No evidence for visuotopographically organized connections of PLγ was found. It should be noted that recent physiological studies have shown that inferior temporal cortex does not contain a detailed visuotopic map. The lack of reciprocity between PLγ and prefrontal cortex was further underscored in our HRP study where no prefrontal positive targets were found in PLγ after HRP injections in prefrontal cortex. Peroxidase positive cells were found in PLγ after HRP injections in areas 20 and 21. Thus, PLγ is likely to be associated with associational cortex with inferotemporal cortex and thereby establishes a cortico-thalamicortical 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 Research Board and Biomedical Research Support Grants)


In view of the current interest in the tripartite 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 distribution of the retinal input to the hamster's superior colliculus (SC), and (2) to correlate afferent conduction velocities with the responses properties of retinal cells. The experiment was accomplished by employing standard extracellular recording techniques in the SC and electrical shocks delivered to the contralateral optic nerve (ON) and optic chiasm (OC). In an additional series of animals, we attempted to delineate the nature of the retinal input to the ipsilateral SC. Here the ON electrode was positioned behind the eye ball ipsilateral to the SC from which we recorded.

Cells driven reliably by shocks delivered to the ON or OC 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 lamine 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 binodal with a broad peak centered at 6 m/sec. A few retinal 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 (Baker, et al., 1976, JCN, 168, 439-458), suggest that all retinal ganglion cell types in this species project to the colliculus.

A recent conduction velocity was closely related to a directional selectivity. Ninety percent of the retinal neurons receiving inputs from axons having CV's of less than 5 m/sec were directionally selective while only 42% of those receiving inputs from more rapidly conducting fibers (>5 m/sec) exhibited selectivity.

We tested 116 cells in the anterior portion of the colliculus for the sensitivity to stationary stimuli. Of these, 61% were responsive to ON shock. These electrically responsive 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 COLICULUS. Barry J. Richmond and Robert H. Wurtz. NIMH, Bethesda, MD 20892.

In the monkey superior colliculus some cells show a suppression of activity following saccadic eye movements due to a cortical correlate of the saccadic eye movement. This effect has been observed in the visual areas II, IV and V of the superior colliculus (Chalupa et al., J. Neurophysiol., 1974). 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 inferior temporal cortex of the superior colliculus to see if visual masking is also prominent in this structure.

We tested 116 cells in the anterior portion of the colliculus for responsivity to photic stimulation delivered to the ipsilateral retina. We used both stationary and moving stimuli. When the stationary stimulus was placed in the excitatory receptive field center, the response to the sweep increased markedly. We have not determined whether the effect on the sweep was due simply to the adjacent surround or to a more complex visual interaction 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 (900º/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 was due simply to the adjacent surround or to a more complex visual interaction in cells of cat superior colliculus (Rizzolatti et al., J. Neurophysiol., 1974).

Supported by National Eye Institute (HCA 702072).
THE EFFECT OF ADAPTING TARGET LOCATION UPON THE GAIN OF THE SURROUND RESPONSE MECHANISM OF X CELLS IN CAT RETINA.

2061


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 target locations were more pronounced in the central 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 on-inhibition measure — the off-discharge to an annulus flashed alone in the RF center — the on-inhibition measure — and the magnitude of the off-discharge to a flashing spot in the RF center — the off-excitation measure. The greatest reduction in the gain of the SM was curvilinear for the on-inhibition measure but linear for the off-excitation measure. The relationship between adapting target location and the gain of the SM was generally consistent with the assumption that 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-response to a flashing spot 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 periphery of the RF. The results of the experiment using the off-excitation measure do not support this model.


2063

It has been previously reported (Stone, Rowe & Campion, J. Comp. Neurol., in press) that there is 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 correlated with 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 Y-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 retinas. 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 6% of the ganglion cell population in temporal periphery, but only about 46% in nasal periphery. In siamese mean soma diameters are 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 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 the difference is not as marked as that observed between corresponding regions of common retinas. 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 retinas. 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.

COMPLEX PATTERN DISCRIMINATION IN THE ALBINO RAT: ROLE OF STRIATE CORTEX AND THE IPSILATERAL RETINO-CORTICAL PATHWAY.

2062

Laurence A. Rutherfurd and Michael L. Schwieter. Dept. Psychol., GEORGE WASHINGTON UNIVERSITY, WASHINGTON, D.C. 20052

Monocular deprivation in the rat, as in the cat and monkey, produces a variety of morphologic and functional abnormalities which alter the development of normal behavior. A chief advantage of the rat as a model system is the fact that the optic nerves fibers, unlike those of the higher primates, are not 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 fibers of the optic nerve in normal 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; Lasley, Psychol. Rev. 31:136, 1924; Muniz and Sutherland, J. Comp. Neurol. 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 decrement in behavior produced by visual deprivation.

(Supported by NIMH Grant MH RO1-27424)


2064

In the adult cat, both binocular 1st suture and monocular paralysis have been shown to reduce the encounter rate for x-cells (cells characterized solely on the basis of response to optic chiasm shock-OX neurons in the lateral geniculate nucleus (LGN)). In contrast, kitten onsets sutured produces a pattern of Y-cell losses. These contrasting results raise the question of 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 was examined in the rat. 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 (Ay) and in laminae subserved by the paralyzed eye (Aa and C). In contrast, infant onset 1st suture produces no reported effects on the nondeprived laminae.

The left eye of the rat is considered to be innervated by the mobile eye, whereas the right eye is considered to be the nonmobile eye. The left (mobile) eye is involved in the visual system while the right (nonmobile) eye is rather involved in somatic sensation. The effects of monocular paralysis of infants and adults is seen in that the A layer, innervated by the mobile eye, suffers a partial X-cell loss in both instances. In monocular paralysis of parathyroid hormone, 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- and Y-cells are affected. These data further indicate that factors sensitive to the impact of environmental modification are very sensitive to the age of onset of the 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.
TRANSIENT VARIABILITY CHANGES IN CAT RETINAL GANGLION CELLS DURING ADAPTATION TO DIFFUSE ILLUMINATION. Arthur C. Sanderson, Dan Schwebzer-Tong, and Winfried H. 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 change in diffuse retinal illumination. Step changes of one log unit in the range 10^{-4} to 10^{3} cd/m² with a fully dilated pupil (equivalent to a retinal illuminance of about 0.1 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. Model calculations 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 multivariate gain control, for example, through cellular or local circuit mechanisms, rather than adjustments additively by network inhibition.

(Supported by NSF grant ENG75-17536 and NIH Training Grant 5-T32-EY07477.)

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 were 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, whereas the LGN 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. Neurol. 168, 1976). The present results address this controversy.

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 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 speculation of Movshon & Dursteler (1977) or of Cragg et al. (1976), but are consistent with the suggestion of Wiesel & Hubel (1963), concerning the relationship between cortical and LGN changes resulting from monocular vision.
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 & Dept. of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

The time of neuron origin and the development of afferent connections in the monkey's visual system have been examined previously (Rakic, '77) but little is known about the formation of efferent projections from the visual cortex. We investigated the development of the cortical topographic projection 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 (E) 63 to 66 (E69) were injected with tritiated proline (20-40 µCi) and the large intracranial section 0.1-0.2 µCi was injected into the occipital cortex. Each fetus was restored to the uterus until about 24 hours later when it was killed by perfusion, and its brain was processed for autoradiography.

In the autoradiographs of the E63 fetus no radioactive label was present in the contralateral radiations. The LGN and SC were 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 E83, indicating that the topographic 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.

Three different pathways from the vertebrate retinae 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 efferentfferent pathway is topographically ordered prior to the laminogenesis of the LGN and before the segregation of afferents from the two eyes. Supported by N.I.H. grants NS 11233 and EY 05172.

A wiring diagram for the receptive fields of bipolar cells in the generalized vertebrate retina. Robert Siminoff, BRI, UCL, 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—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 red 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 center 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. Additionally, overlapping of the central and surround regions is prohibited. The contralateral LGN is divided into 3 terminal nuclei (dorsal, lateral, and medial), each divides further into 3 terminal nuclei. 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 9-cones of the 3 spectral types of cones. Electrical coupling between like-horizontal cells produces spatial summation so that as the 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 cells. Two types of bipolar cells, A and B, are present in parallel. The HPB were first inputs produce hyperpolarization and the DPBC when direct inputs produce depolarization. Thus, each wiring diagram produces two types of bipolar cell—hyper/dep and dep/hyper.


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 intracellular 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 (20-30º) square, slowly moving patterned textures. 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 0.5-1.5/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 includes a retinal component of the accessory optic system. MTN cells project to the NOT and 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. 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 retinal 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 organization of the three semicircular canals of the vestibular system and that this organization allows for integration of visual and vestibular signals in a single coordinate system. (Supported by PHS Grant HS-13742).
2073 BE HIGHLY CORRELATED WITH THE PERCENTAGE OF RESPONSIVE CELLS AND NONDEPRIVED EYE IN 5 MD CATS. THE MEAN ACUITY FOR RS CATS IS DEPRIVED EYE OPENED AND MDE CATS. THE RESULTS OF THESE ADDITIONS SHORTLY AFTER BIRTH (MDE), ABOUT 68% OF THE CELLS ARE RESPONSIVE TO THE INTIALLY-DEPRIVED EYE FOLLOWING POSTCRITICAL-PERIOD ENucleATION OF THE NONDEPRIVED EYE IN A MD CAT (MD-DE) (KRATZ ET AL., '76; SMITH ET AL., '78). FURTHER, AN OVERALL DERM VARIANCE 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 BHRICAL DECER EPR (BD) ABOUT 50% OF THE CELLS IN THE STRIATE CORTEX ARE RESPONSIVE (KRATZ & SPEAR, '76; PETTIGREW, '74; WIESEL & HUBEL, '65). Finally, IN MD'S WITH THE OTHER EYE REMOVED SHORTLY AFTER BIRTH, ABOUT 64% 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 GRAVE GRATINATIONS (LUMINANCE 13 cd/m²). FURTHER, WE HAVE ESTABLISHED THE VISUAL ACUITY FOR 2 RG, 3 MD-DE, 2 BD AND THE NONDEPRIVED EYE IN 5 MD CATS. THE MEAN ACUITY FOR RG 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 EXPERIMENTS WILL BE PRESENTED. FURTHER, 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. SOOMAO AND LEWIS G. BISHOP. UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES, CA 90007.

WHEN A TETHERED FLYING INSECT SEES RELATIVE ANGULAR MOTION, IT WILL MOVE AS TO NULL OUT THIS RELATIVE MOTION. GOETZ MEASURED FLYING TORQUE PRODUCED IN A TETHERED FLY AND FOUND THAT THRUST RESPONSE WAS MAXIMUM WHEN A HORIZONTALLY ORIENTED STRIPED PATTERN WAS MOVED VERTICALLY; THE TURNOVER TORQUE WAS MAXIMUM WHEN A HORIZONTALLY 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 RESSEMBLES 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. MOVING STIMULUS EVOKES A RESPONSE WHOSE MAGNITUDE IS EQUAL TO THAT EVOKED BY A STATIONARY STIMULUS SEVERAL ORDERS OF MAGNITUDE BIGGER. 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 FOREPaw OR BE SHOCKED ON ITS ON SAFE 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 IN 3 GROUPS. THE KITTENS LEARNED THE TASK EVENTUALLY. AT 13 WEEKS OF AGE, SINGLE CELLES WERE RECORDED FROM POSTCRUSSY Gyrus (PC), PRIMARY VISUAL CORTEX (V1) AND VISUAL ASSOCIATION CORTEX (VASCX). PC SHOWS A GREATLY ENLARGED FOREARM LOCUS FOR THE TRAINED SIDE 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 PREPERENCE OF CELLS TO PREFER ORIENTATIONS USED DURING TRAINING AND OFTEN RESPOND TO FORARM STIMULI. SURPRISINGLY LARGE EFFECTS (CONSIDERING THE BREVITY OF VISUAL TRAINING COMPARED WITH TOTAL VISUAL EXPERIENCE) ARE PRESENT IN V1: 1) A LARGE SHIFT TOWARDS MONOCULARITY; 2) ORIENTATIONS USED DURING TRAINING ARE SIGNIFICANTLY MORE REPRESENTED THAN OTHERS; 3) MOST IMPACTFUL, THE PROPORTION OF CELLS TO UNUSUAL STIMULI IN NORMALLY REARED KITTENS. NO ATROPHY OF ANY KIND EXISTS IN THOSE 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 DEVELOPMENT OF VISUAL CAPABILITIES AND QUALITATIVE EXPERIENCES. THESE RESULTS ARE SIGNIFICANT NOT ONLY BECAUSE OF WHAT THEY TELL US CONCERNING HOW EXPERIENCES ARE STORED IN THE BRAIN, BUT (AS WE BELIEVE) THAT THESE CHANGES OCCUR IN DEEP AND PERMANENT BECAUSE OF THE IMPLICATIONS THEY CARRY CONCERNING EARLY EXPERIENCES IN HUMAN CHILDREN.

SUPPORTED BY RESEARCH GRANTS FROM NIH (EYO 7005) AND CNS, ROME, (70.0168/18).

2076 EFFECTS OF SELECTED LESIONS IN VISUAL CORTEX ON INTERHEMISPHERIC TRANSFER OF FORM DISCRIMINATIONS IN CATS. JAMES M. SPRAUGE, GIOVANNI BERLUCCHI, ALESSANDRO ANTONINI AND ALFIO SIMONE. DEPT. ANAT. SCH. MED., UNIV. PENNSYLVANIA, PHILADELPHIA, PA 19104 AND INST. PHYSIOI., UNIV. PISA, 56100, PISA ITALY.

Suprasylvian lesions removing cortical areas 7 and 21, and portions of area 17 of the left hemisphere of suprasylvian area (LSA) were placed unilaterally in split-chiasm cats. By comparison with the nonlesioned 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 a unilateral 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 LHA 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 discrimination 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 anterior-medial and posterior-medial portions of LSA (Clar-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 of the posterior limen and of the lateral suprasylvian region of the left hemisphere (areas 17 and 18) showed good interhemispheric transfer of monocularly learned pattern discriminations. The capacity for interocular transfer of these discriminations was 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 (EYO 7005) AND CNS, ROME, (70.0168/18).

The substantia gelatina 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. 69:1, 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. [3H]-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 133 neurons in a patch of lamina A and found that the labeled cells formed about 22% of the population. They were the smallest cells, (12-19µm) and lacked the multilaminar body which is the hallmark of the somewhat larger (16-24µm) class II relay cell (Jahn in the K. J.C., 172:255, 1977). In EM autoradiograms the F-terminals were intensely labeled while optic terminals (ONF) 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 geniculate, 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 007008-28)

**2078** A SYNAPTIC TRIAD AS THE INPUT TO "X" LATERAL GENICULATE CELLS. John K. Stevens, George L. Gerstein, Dept. Physiology, Univ. of Penn., Phila., PA. Using single electrodes we have recorded from 26 unit-pairs in cat lateral geniculate nucleus (LGN). In each case the electrodes consisted of a small nicotinic positive action potential (AP), followed by a larger biphasic AP. The small AP has been identified via electrophotial stimulation as an excitatory action potential and, by exclusion we assume the large AP represents an LGN cell. Crosscorrelograms between these OT fibers and LGN cells were studied in the present release and P. After a single optic tract AP the probability of finding an LGN AP was enhanced for 1.0 to 1.5 msec (1st period). This 1st peak in the crosscorrelogram was followed by a decrement in the firing probability of the LGN cell for a 1.0 to 2.0 msec period (P period). The P period was followed by a turn by a second increment in the firing probability of the LGN cell (2nd peak). Special pattern crosscorrelograms demonstrate that these two components are contingent only upon a single AP from the OT fiber and not on the firing of the LGN cell. Thus, the P period and 2nd peak do not represent OT fiber and LGN cell to OT fiber and LGN cell. These findings are inconsistent with models of synaptic triad, where an OT fiber would excite both a LGN cell and an interneuron. The interneuron would then inhibit the LGN cell to produce a 1st peak. The 2nd excitatory peak would represent a rebound effect. We observed response planes on 18 pairs. In 17 of these both the OT fiber and LGN cells were recorded, and for an OT-LGN pair had homogeneous response planes ("X" cells). The remaining OT-LGN pair had homogeneous planes ("Y" cells). On six occasions cells failed to record for the LGN cell, but the remaining 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 circuitry 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 P terminals in the triad which accumulate DABA are derived from inhibitory interneurons. (Abstracts in this volume. Supported by NIM grants 1E01832 and NS05660.)


Responses from single ganglion cell fibers in the optic tract of cats were recorded extracellularly with lacquer 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 varied in several experiments. Responses to off-center X were well maintained by background luminance and larger spot diameters. The waveform did not significantly influence their following rates at higher frequencies or the peak frequency response. On-off responses to off-center X were consistent with responses to squarewave modulated stimulus could be predicted from the response to squarewave modulated stimulus could be predicted from the response to squarewave modulated stimulus. 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 L52. The "splenial visual area" of Kalls and Whitmore (J. Neurophysiol., 232:275-283, 1973) was labeled after injections into P and lateral portions of LPI but not after injections into LPI or medial portions of LPI. Area 7 is labeled after injections into LPI but not into LPI or LPI. Area 19 is labeled after injections into LPI and P but not LPI. Area 20 appears on the basis of both electrolytic and extracellular recordings to consist of two subdivisions, and both of these are labeled after injections into LPI, LPI and P. We conclude that Venkatesh's data suggests that the posterior zones is largely represented by the uniqueness of their projections to identified cortical areas. (Supported by Grants NS 02453 from NSF, and T32 ET 07035-02 from NIMH.)


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 mediodorsal to lateral in the dorsoventral plane. Using the scheme and nomenclature of Updyke (J. Comp. Neurol., 173:81-122, 1977), we have made injections of tritiated amino acid into the LGN (1) lateral posterior thalamus (LPi) and superior lateral posterior thalamus (LPl), (2) lateral posterior thalamus (LPi), and pulsivar (P). LPI corresponds to the tegmentum-projecting regions of the LGN, P to the ponto-tegmental region. The patterns of cortical connectivity of these thalamic subdivisions were related to the electrophysiological and anatomical findings of the visual areas of the cat cortex. In the suprasylvian cortex (Palmer, et al., J. Comp. Neurol., 177:237-256, 1978), areas VLM and VLS are related to lower LPl but not to LPI or P. Injections of the AMLS appear 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 LPi. 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 L52. The "splenial visual area" of Kalls and Whitmore (J. Neurophysiol., 232:275-283, 1973) was labeled after injections into P and lateral portions of LPI but not after injections into LPI or medial portions of LPI. Area 7 is labeled after injections into LPI but not into LPI or LPI. Area 19 is labeled after injections into LPI and P but not LPI. Area 20 appears on the basis of both electrolytic and extracellular recordings to consist of two subdivisions, and both of these are labeled after injections into LPI, LPI and P. We conclude that Venkatesh's data suggests that the posterior zones is largely represented by the uniqueness of their projections to identified cortical areas. (Supported by Grants NS 02453 from NSF, and T32 ET 07035-02 from NIMH.)
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.

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 within 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 task. 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 number of trials. The degree of interocular transfer was assessed, but either the pattern used on 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 EC group was much better (index of interocular transfer derived from Murdock's formula: 0.93) than in the EI group (index of interocular transfer derived from Murdock's formula: 0.63) using visual cortical lesions excluding the suprasylvian area. The authors concluded that transfer must depend on other cortical areas within the suprasylvian area. It was found that interocular transfer for the newly learned patterns was measured. It was found to be very poor in either direction (index of transfer: 0.20). The authors speculated that transfer may depend 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.
Thus, although interactions with striate cortex are critical for apparently inputs from central vision are especially important neurons, may reflect differences in the cortical processing required for spatial vs. object vision.

The relative effects of the striate and extrastriate lesions in the two studies were reversed, suggesting that posterior parietal cortex is more dependent on ipsilateral striate inputs than is inferior temporal cortex. However, the two are organized differently: relative to inferior temporal gyrus, the posterior bank of the superior temporal sulcus, and extrastriate occipital cortex (cf. Van Essen and Zeki, J. Physiol., 259, 525-554, 1976), the distribution of callosal-projection neurons may provide a useful morphological feature should respond differently from conventional relay cells. An additional population of pyramidal in shape. An additional population of pyramidal cells also possess presynaptic dendrites; a feature which would imply additional "interneuron-like" functions.

In an attempt to identify the specific cells of origin of the pretecto-olivary pathway, we injected horseradish peroxidase (HRP) into the pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

In our studies we have used the projection 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, the pretectum, projects to the dorsal accessory olive in both the cat and the rat. Although Olshausen and Hebb (1968) concluded that the pretectum projects to the lateral accessory olive, our projection data show that the pretectum projects to the medial accessory olive of the cat. The projection is especially dense in the tree shrew. Thus far, we have been able to identify the specific cells of origin of the pretecto-olivary pathway, we injected horseradish peroxidase (HRP) into the pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

The projection of the pretectum upon the inferior olivary complex is an autoregulatory and horseradish peroxidase analysis in the tree shrew, the rat and the cat. J. T. Weber, M. F. Huerta, M. Behn, G. J. Royce and J. K. Harting. Department of Anatomy, University of Wisconsin, Madison, Wisconsin, 53706.

In our studies we have used the projection of visual inputs to the inferior olivary complex. 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 pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

In our studies we have used the projection of visual inputs to the inferior olivary complex. 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 pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

In our studies we have used the projection of visual inputs to the inferior olivary complex. 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 pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

In our studies we have used the projection of visual inputs to the inferior olivary complex. 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 pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

In our studies we have used the projection of visual inputs to the inferior olivary complex. 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 pretectal complex of one rat. Following large olivary injections in the cat, backfilled 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.

In our studies we have used the projection of visual inputs to the inferior olivary complex. 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.
Anatomy, Vanderbilt University, Nashville, TN 37240.

HRP injections in striate cortex resulted in HRP-positive cells. Most of the labeled cells were in layer VI, and many of these were characterized by having reciprocal connections with striate cortex. Furthermore, Ungerleider and Mishkin (Anat. Rec. 1982) have recently reported that the monkeys of projects 1 and 2 received multiple injection sites in striate cortex. Depts. of Psychology and Neuroscience, Vanderbilt University, Nashville, TN 37240.

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 extended into Area 18). Retrograde labeling 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 labeled cells were seen in layers II and III. Labeled cells 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 the olfactory cortex were labeled.

The results show that a region of STS in macaque monkeys is reciprocally connected with striate cortex in a pattern similar to that observed in New World monkeys and primates. We conclude that a region of STS in Old World monkeys is homologous to that of the New World monkey.

Supported by NIH Grant EY-02666.

The number of vesicle-containing profiles and synaptic densities in the parvo- and magnocellular laminae and interlaminar zones of the dorsolateral geniculate nucleus of Macaca monkeys. James R. Wilson and Anita E. Hendrickson. Dept. of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

The number of vesicle-containing profiles and synaptic densities in the parvo- and magnocellular laminae and interlaminar zones are counted and their relative frequencies determined in normal adolescent monkeys. Comparable counts were also made in the LGN of adult albino monkeys deprived from 2 weeks postnatally by monocular lid-stabilization. A large region of labeled cells in STS of about 10 mm in width. Most of the labeled cells were in layer VI, and many of these were characterized by having reciprocal connections with striate cortex. Occasionally, HRP-positive neurons were also found in layer V, and a few labeled cells were encountered in layer III. In addition, a few labeled cells were seen in layers II and III. Labeled cells 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 the olfactory cortex were labeled.

The results show that a region of STS in macaque monkeys is reciprocally connected with striate cortex in a pattern similar to that observed in New World monkeys and primates. We conclude that a region of STS in Old World monkeys is homologous to that of the New World monkey.

Supported by NIH Grant EY-02666.

The number of vesicle-containing profiles and synaptic densities in the parvo- and magnocellular laminae and interlaminar zones of the dorsolateral geniculate nucleus of Macaca monkeys. James R. Wilson and Anita E. Hendrickson. Dept. of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

The number of vesicle-containing profiles and synaptic densities in the parvo- and magnocellular laminae and interlaminar zones are counted and their relative frequencies determined in normal adolescent monkeys. Comparable counts were also made in the LGN of adult albino monkeys deprived from 2 weeks postnatally by monocular lid-stabilization. A large region of labeled cells in STS of about 10 mm in width. Most of the labeled cells were in layer VI, and many of these were characterized by having reciprocal connections with striate cortex. Occasionally, HRP-positive neurons were also found in layer V, and a few labeled cells were encountered in layer III. In addition, a few labeled cells were seen in layers II and III. Labeled cells 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 the olfactory cortex were labeled.

The results show that a region of STS in macaque monkeys is reciprocally connected with striate cortex in a pattern similar to that observed in New World monkeys and primates. We conclude that a region of STS in Old World monkeys is homologous to that of the New World monkey.

Supported by NIH Grant EY-02666.


The number of vesicle-containing profiles and synaptic densities in the parvo- and magnocellular laminae and interlaminar zones of the dorsolateral geniculate nucleus of LGN were counted and their relative frequencies determined in normal adolescent monkeys. Comparable counts were also made in the LGN of adult albino monkeys deprived from 2 weeks postnatally by monocular lid-stabilization. A large region of labeled cells in STS of about 10 mm in width. Most of the labeled cells were in layer VI, and many of these were characterized by having reciprocal connections with striate cortex. Occasionally, HRP-positive neurons were also found in layer V, and a few labeled cells were encountered in layer III. In addition, a few labeled cells were seen in layers II and III. Labeled cells 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 the olfactory cortex were labeled.

The results show that a region of STS in macaque monkeys is reciprocally connected with striate cortex in a pattern similar to that observed in New World monkeys and primates. We conclude that a region of STS in Old World monkeys is homologous to that of the New World monkey.

Supported by NIH Grant EY-02666.


Unilateral lesions of the superior colliculus in cats have been reported to produce ipsiversive circling, which has been attributed to destruction of neurons in deeper layers of the colliculus which project through the tectospinal tract to the reticulotegmental area of the brainstem. In the present study, we have stressed that lesions interrupting ascending catecholamine pathways in the brainstem are also associated with post-operative circling. Because the cat has a well-developed midbrain tegmentum subjacent to collicular neurons from which the tectospinal tract arises, and because lesions of the superior colliculus frequently extend into this tegmentum, this study was designed to test the hypothesis that ipsiversive circling following unilateral ablation of the superior colliculus is due to interruption of synaptic efferents from the colliculus but that 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 atrophy of diencephalic origin (ipsilateral to the lesioned colliculus). For each cat, behavioral tests, histological confirmation and biochemical assays of levels of activity in forebrain of the biosynthetic enzymes for dopamine and norepinephrine were performed independently. As in previous "between-strains" comparisons of Sprague-Dawley rats vs. pigmented rats of other strains, there was no way to show that the monkey strain was involved in this present study, as in previous "between-strains" comparisons of Sprague-Dawley rats vs. pigmented rats of other strains, there was no way to show that the monkey strain was involved in this present study.