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

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Indexes

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AGING
1 DAILY HIGH-FREQUENCY STIMULATION DIFERERENTIALLY PROLONGS SYMPATHETIC ENHANCEMENT IN MIDDLE-AGED AND SENESCENT RAT HIPPOCAMPUS. G. A. Barnes and R. T. Bartus. Psychoneuroendocrinology, 4, 1979, 205-231. The perifornical—pericerebral cell syanapse in the hippocampus has been shown to underlie long-term potentiation (LTP) after brief high-frequency bursts of stimulation (Bliss & Lomo, 1973). Barnes (1979) found that the maximum extent of enhancement, following a single stimulus session, was equivalent in middle-aged and old rats. The decay time constant, however, was extended from 2 to 5 days in the young animals by repetition of the enhancing stimulus at 24 hour intervals for three days. The time constant for the old rats failed to show an increase. In the present study we sought to determine whether the decay in enhancement in old rats could ultimately be extended given a sufficient number of repetitions of the enhancing stimulus. A second goal was to determine whether the time constant of enhanced animals tended towards some asymptote or whether it was extended indefinitely with further repetition.


5 Old (mean age = 40 mos.) and young (mean age = 6 mos.) New Zealand albino rabbits were exposed to Pavlovian conditioning training in which corneoretinal potential (CRP), heart rate (HR), and electromyographic (EMG) conditioned reflexes (CRs) were assessed. Three experiments were performed in which 75 db tones served as CSs and a 300 msec, 3 ma paraorbital electric shock served as the US. Two experiments assessed single periods of age-related short-term memory impairments in relation to cellular changes in specific neuronal circuits that may be involved in the mediation of short-term memory impairment in middle-aged and senescent rabbits. Animals with electrodes that failed to remain stable for the full period of observation were eliminated from the final data analysis. The stimu stimulus current was set just above the threshold for granule cell discharge and was kept constant throughout the experiment. After baseline control responses were obtained, 12 high-frequency (150-1000 Hz) stimuli were given (single pulse burst of 20 stimuli) at 24 hour intervals. The response amplitude was measured prior to and following each high-frequency session, and at daily intervals for three weeks after the last (12th) session. Synaptic enhancement reached an asymptotic value of approximately 60% in both age groups. The decay time constants reached maximal values of 37.3 days for young and 29 days in the old groups, respectively. The rate of growth of the time constant was a decreasing function of the number of stimulus repetitions.

6 We conclude from these data that the decay time constant of synaptic enhancement in the *ascia dentata* is considerably prolonged by daily repetition of the enhancing stimulus and that the process underlying the increase in the decay time constant, rather than enhancement per se, is deficient in senescent rabbits. Furthermore, it does not appear that enhancement can be permanent by any reasonable amount of stimulus repetition.

7 A striking effect was observed on retention of the inhibitory avoidance task. In fact, mice fed the choline-enriched diet performed as well as young, 3 month old mice, while mice fed the choline-deficient diet performed as poorly as senescent mice (23 months and older). Preliminary biochemical analyses of the mouse brains demonstrated that significant differences in specific pre- and post-synaptic markers existed between the choline-enriched and choline-deficient groups. More detailed results will be presented. These data demonstrate that chronic manipulation of the cholinergic system, via precursor control, can produce significant changes in brain and behavior in middle-aged mice. Further, these changes are similar to those that occur normally in aging suggest that certain debilitating effects of age may, therefore, be modulated through appropriate precursor control.
AGING

5 NEURAL INCREASE IN VARIOUS REGIONS OF THE FISH BRAIN. R.R. Coggshall, R.B. Leonard, and G.C. Birge. Departments of Anatomy and of Physiology and Biophysics, The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

It is generally accepted that the number of neurons in most parts of the vertebrate nervous system is relatively constant in postembryonic life. It is clear in teleosts, however, that there is a constant addition of ganglion cells to the retina for as long as the animal lives. In elasmobranchs there seems to be a constant increase in dorsal root ganglion cells and axons and ventral root axons. This leads to the question as to whether neurons in all, or almost all, parts of the nervous system in lower vertebrates increase in number throughout the life of the animal. The present study is an attempt to obtain at least a partial answer to this question. Guppies (Lebiasites) at various ages (Table I) were serially sectioned in paraffin and 1) the Purkinje cells of the cerebellum, 2) the neurons in the nucleus gloverous, 3) the dorsal root ganglion cells of the 2nd dorsal root ganglion and 4) the cells in the ventral horn of the 2nd segment were counted. The data are presented in Table I.

<table>
<thead>
<tr>
<th>AGE IN DAYS</th>
<th>1</th>
<th>4</th>
<th>10</th>
<th>23</th>
<th>38</th>
<th>61</th>
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<tr>
<td>Pur. Cells</td>
<td>1706</td>
<td>1493</td>
<td>3036</td>
<td>----</td>
<td>3398</td>
<td>4558</td>
<td>5606</td>
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<tr>
<td>Nuc. Glom.</td>
<td>2199</td>
<td>2447</td>
<td>2886</td>
<td>4651</td>
<td>4620</td>
<td>7017</td>
<td>9153</td>
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<tr>
<td>D.R.G. Cells</td>
<td>330</td>
<td>372</td>
<td>667</td>
<td>670</td>
<td>433</td>
<td>910</td>
<td>1258</td>
</tr>
<tr>
<td>V.H. Cells</td>
<td>966</td>
<td>980</td>
<td>921</td>
<td>----</td>
<td>1090</td>
<td>1375</td>
<td>1640</td>
</tr>
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</table>

These preliminary data suggest that there is a relatively steady increase in neuronal numbers in all of these areas. This would seem to imply that there is an increase in neural numbers in most parts of the brain in adult fish and in this respect, fish would seem to differ markedly from mammals. It is possible that this difference is related to the greater capacity of fish for neural regeneration as compared to mammals. This work is supported by NIH grants NS 07377 and NS 10161.

6 EFFECTS OF ENVIRONMENT AND SOCIAL INTERACTION ON LEARNING AND MEMORY IN SENESCENT AND MATURE MICE. Dennis D. Crady* and Elton E. Quinton. Dept. Psych., Univ. of Louisville, Louisville, Kentucky 40208.

The processes of learning and memory are believed to be dependent upon protein synthesis. Senescence has been hypothesized to result from a chronological decrement in efficiency of protein synthesis in the brain. If these processes are truly basic, one might expect to see a decrement in learning and memory abilities in senescent animals as well as an exaggerated impairment following protein synthesis inhibition. This possibility interacts between protein synthesis inhibition, learning and memory processes, and aging.

Three age groups of C57Bl/6J mice (7-10 months, 28-31 months, and 35-36 months) were trained on a simultaneous five-choice visual discrimination active avoidance task. Animals were given 10 consecutive 10 day sessions on the task. Animals were given Anisomycin (5 mg./kg.) or water was administered 5 minutes prior to the start of each daily session. Animals completed the session within 25 minutes of the drug administration. All groups demonstrated a high degree of acquisition over the 10 sessions. The performance of the mature control group was superior to that of the senescent control group. Anisomycin significantly impaired the performance of the mature mice, but had no apparent effect on the performance of the senescent mice (compared with the control group).

These data suggest that the hypothesized learning deficit in the senescent animals, but it was not clear that this deficit was a result of a decline in protein metabolism. An impairment in learning was demonstrated in the mature drugged group which was not similarly reflected in the senescent drugged group. Hence the hypothesis differentiated effect with a greater impairment in the senescent group was not obtained. These results suggest a differential effect of anisomycin on protein synthesis in mature and senescent mice.

7 SYNTHESIS OF MONOAMINE OXIDASE IN MICE AS RELATED TO AGING. NEURAL INCREASE IN VARIOUS REGIONS OF THE FISH BRAIN. R.E. Young. Departments of Anatomy and of Physiology and Biophysics, The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

At nine days about 40% of the activity had returned. At 26 days after treatment, the recovery of activities, and thus synthesis, between young, middle age, and old mice. Our findings indicate that old mice can synthesize both forms of MAO at the same rate as young mice. The processes of learning and memory are believed to be dependent upon protein synthesis. Senescence has been hypothesized to result from a chronological decrement in efficiency of protein synthesis in the brain. If these processes are truly basic, one might expect to see a decrement in learning and memory abilities in senescent animals as well as an exaggerated impairment following protein synthesis inhibition. This interaction between protein synthesis inhibition, learning and memory processes, and aging:

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In interest of defining the behavioral impairments associated with age, and the biochemical sources of these impairments, has been undertaken recently. It was found that brains of young adult rats, that had been depleted of muscarinic receptors in the medulla, exhibited decreases in the length of neuronal plasma membrane. The membrane length was estimated with the help of a map-measurer.

In contrast, no age-related differences were observed in the length of neuronal plasma membrane. The membrane length was estimated with the help of a map-measurer. However, the number of axon terminals contacting membranes of longitudinally cut dendrites was found to be significantly decreased in young adult rats compared to senescent rats. These results suggest that a loss of presynaptic terminals underlies synaptic changes associated with age.


Severe dietary restriction throughout adult life has been reported to significantly enhance the longevity of rodents in the laboratory. In order to evaluate the effect of longevity-promoting dietary regimens on the age-related changes in rat brain, lipofuscin accumulation in cerebellar purkinje cells was measured in three dietary treatment groups: rats reared in large litters (16 pups) and fed ad lib following weaning at 21 days of age (group HH); rats reared in three dietary treatment groups: 1) rats reared in large litters (16 pups) and fed ad lib following weaning at 21 days of age (group HH); 2) rats reared in large litters (16 pups) and fed ad lib following weaning at 21 days of age (group HH); 3) rats reared in large litters (16 pups) and fed ad lib following weaning at 21 days of age (group HH).

Comparison of the number of intersections of a grid reticle which overlay autofluorescent lipofuscin granules in a single plane of focus. Perikaryal volume was estimated in a similar manner using phase contrast microscopy. In comparison with group HH, groups HL and LH exhibited significantly less lipofuscin (t < .05, one-tail test). However, group HH exhibited a significantly lower perikaryal volume than group HH. Consequently, there were no significant group differences in the rate of accumulation of lipofuscin granules.

If these preliminary results are confirmed by additional data, it would imply that restricted rats are undergoing age-related changes in brain structure and function.


A loss of axo-dendritic and axo-somatic synapses was described by us earlier in the dentate gyrus of senescent rats (Neurosci. Abstr., 1977; 3: 106; 1978; 4: 113). This loss of synapses was determined in relation to the postasymptotic elements remaining in senescence, and it could, therefore, be due to a disappearance of synaptic densities or to a loss of presynaptic terminals.

In the present study, an attempt has been made to elucidate the question whether a loss of presynaptic terminals underlies age-related synaptic changes. Male rats of the Fischer-344 strain were used. A group of five senescence rats of 25 months of age was compared with a group of five young adult rats of 3 months of age. After perfusion of animals with Karnovsky's fixative, the rostral portion of the left dentate gyrus was examined in transverse sections of the entorhinal area. To label somata of granule neurons, some sections were processed with a combination of anti-acetylcholinesterase and anti-calcium antibody. The number of axon terminals was counted and expressed per unit length of neuronal plasma membrane. The membrane length was estimated with the help of a map-measurer. Examination of axon terminals in contact with neuronal soma membranes showed that their number was significantly decreased in the group of senescent rats. An increase in the number of axon terminals was found to be associated with any significant change in the length of neuronal plasma membrane in the dentate gyrus. However, the number of axon terminals was significantly lower than that of the observed age-related loss of axo-somatic synapses, and further work is in progress to determine whether the same is true for the age-related loss of axo-dendritic synapses.

Supported by NIA grant AG 000779.
AGING OF CHOLINERGIC SYNAPSES IN AUTONOMIC GANGLIA AND IRRIS OF THE CHICK. Ezio Giacobini, Mario Marchi*, and Douglas Hoffman*. Lab. of Neuropsychopharmacology, Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, Ct. 06268.

Information of the aging process of cholinergic synapses in the peripheral nervous system is elusive. We followed the pattern of variation in the endogenous levels of acetylcholine (ACh) and choline (Ch) in sympathetic (lumbar) ganglia, a parasympathetic (ciliary) ganglion and its target organ, the iris, from 1 to 18 years of age in the chick. The radioensymatic micromethod of McCaman and Sletzler (J. Neurochem. 26: 669, 1976) was used. The data are expressed as pmoles per ganglion or iris. In the iris, ACh levels remain constant between 1 and 1.7 yrs. (402 ± 42 to 488 ± 29). Following this period ACh levels decrease continuously from 289 ± 27 to 198 ± 40 at 5 yrs., and to 68 ± 11 at 7 yrs. At this latter age the amount of ACh is less than in the iris at 14 days of incubation (d.i.). The ACh content in the sympathetic ganglion is unvaried between 1 and 2 yrs. (182 ± 47 to 259 ± 42) and decreases slightly at 5 yrs. (130 ± 29) with no further decrease up to 7 yrs. (155 ± 48). The ACh levels of the ciliary ganglion show a different pattern. The ACh content increases > 2 fold (161 ± 40 to 384 ± 89) between 1 and 2 yrs., then declines at 3 yrs. (236 ± 60) with the value at 5 yrs. being the same as at 1 year of age. This slight fall continues up to 7 yrs. (96 ± 13). The Ch content parallels the ACh pattern in all three organs. The V_max for Ch uptake follows the same pattern as ACh levels. This is the same trend we observed during development (Marchi et al., 1979, abstract, this meeting).

Conclusion: 1) Unexpectedly, the ACh content of the ciliary ganglion continues to increase during the first part of aging (1-3 yrs.); whether or not these biochemical changes correlate with morphological changes in the maturing synapse remains to be seen. 2) On the contrary, ACh levels in the iris decrease during aging, reaching embryonic levels at 7 years. If these values are calculated per protein the ACh level of the iris is lower at 7 years of age than at 5 d.i., suggesting that low levels of ACh may be insufficient for synaptic function at this age or that decline of function occurs. 3) Ch uptake seems to be a limiting factor in regulating ACh synthesis during aging as it is during development. Changes in V_max closely parallel changes in ACh levels.

13


Aging female rats show a gradual cessation of regular ovulatory cycles. Initially this acyclicity is characterized by a state of constant estrus (CE) as revealed by persistent vaginal cornification throughout the estrus cycle. Subsequently a state of persistent diestrus (PD) ensues interrupted by estrous cycles at irregular intervals. Since changes in prolactin secretion are seen in young females during periods of acyclicity (e.g., pseudopregnancy), the present study was undertaken to examine prolactin secretory patterns in aged PD and CE rats.

Female Long-Evans rats 20-28 months old were housed in standard animal facilities with an alternating light cycle of 14-hr of light (0500-1900 hr) and 10-hr of dark. Each animal was fitted with a chronic intrastriatal cannula and allowed to recover for 2 days. Blood samples (0.15ml) were then obtained via the cannula every 3-hr for 24-hr. The 24-hr program of blood sampling was repeated for each animal 2 or 3 times over a 6 day period, and plasma prolactin was measured by radioimmunoassay using the NIAMD kit and RP-1 standard.

Female rats which are free of pituitary tumors and other gross pathology exhibited two distinct prolactin surges each day. The diurnal surges were relatively small with mean peak prolactin values of 90 ± 9 (SE) ng/ml at 1000-1700 hr. The nocturnal surges were larger, showing peak titers of 389 ± 56 ng/ml at 0200-0500 hr. CE females without estrus showed both diurnal and nocturnal prolactin surges. In contrast to findings in PD rats, however, the diurnal surges were larger (434 ± 179 ng/ml) than the nocturnal surges (174 ± 29 ng/ml). In PD and CE animals which had evidence of pituitary tumors, plasma prolactin values were elevated and did not show any consistent patterns. These findings reveal that aged female rats exhibit daily prolactin surges which are comparable in timing, and for PD rats also in amplitude, to young pseudopregnant females. The prolactin surges in the aged rats, however, differ from those of pseudopregnancy in that they appear to be generated spontaneously and do not require cervical stimulation for activation. (Supported by grants from NIMH and The Ford Foundation).

15 BRAIN UPTAKE INDEX FOR CHOLINE IN AGED RATS. P. Hicks*, C. Rolsten*, L. Hsu*, J. Schoolar, and T. Samorajski. Texas Research Institute of Mental Sciences, Houston, Texas 77030.

Recent studies indicate that there are cerebral vasculature changes with increasing age. There is loss of capillary endothelial cells, and increased capillary length and volume with age (Bar, Adv Neurol. 1: 1, 1972). However, the functional integrity of the blood-brain barrier (BBB) in old animals has not been characterized. Supporto, et al (J Gerontol 34: 162, 1979) did show minimal increased permeability of the BBB with age; however, no information is available in aged animals on the integrity of the BBB carrier systems characterized by Oldendorf (Crit Rev Biochem). Rapoport, et al (J Gerontol 34: 162, 1979) did not report by grants from NIH and The Ford Foundation).

16 FINE STRUCTURAL EFFECTS OF AGE ON THE RAT PINZAL GLAND. John R. Johnson Jr., National Institute on Aging, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Md. 21224.

The pineal is considered to be an endocrine gland having hormonal influences on the testes, ovaries, thyroid, pituitary and adrenals. It is thought that the pineal gland regulates, decreases or inhibits general somatic development and, specifically, the reproductive system. Recently data have accumulated suggesting that the pineal may play a role in the aging process by modulating feedback sensitivity of the hypothalamus. Since hypothalamic feedback sensitivity has been shown to be reduced with increasing age it was hypothesized that the aging pineal would show marked structural alterations.

Female Sprague-Dawley rats 4 mos, 4 mos and 28 mos of age, and female Sprague-Dawley rats 9 mos and 23 mos of age, were sacrificed by aldehyde perfusion and the pineals processed for electron microscopy. With advancing age it was found that the pineal capsule thickness increased and severe collagen infiltration was noted. Accompanying the collagen infiltration were patches of granular material and filaments. Definitive light, dark and intermediate density pinealocytes were present, and no consistent change in their relative frequency was found with increasing age. The number of pinealocytes with deep nuclear invaginations increased with advancing age in the male rats but no pattern was found in the females. Nuclear inclusions, possibly the "kernehulen" reported by Dimitrova in 1901, were identified in the pinealocytes. These inclusions consist of filaments 10nm in diameter and increased in frequency with advancing age. Cytoplasmic dense bodies increased in frequency as function of age. The dense bodies have the structural characteristics of lipofuscin. The maximum diameter of lipid droplets tended to increase with age suggesting the possible role that rate of secretion may decline in older animals. Occasional cells contained reticulated mitochondria and some cell processes had a similar appearance to neuronal dystrophia seen in the dorsal columns of the brainstem in aging animals of several species.
The frequency and amplitude of miniature endplate potentials (MEPPs) exhibit large changes between young and old (30-33 mo) rats (Gutmann et al., J. Physiol. 216:331,1971; Vyskocil & Gutmann, Experientia 28:280,1972). These experiments, conducted at 20°C, showed that the changes (increased amplitude, decreased frequency) were greater in the soleus muscle than in the EDL. In the soleus, MEPP amplitudes slightly increased from 0.56mV to 0.69mV (p<0.01), but there was no significant change in frequency from the value of 5.2Hz in younger mice.

These results differ from those found by Gutmann et al. in that the soleus shows little change, and even in the EDL, the changes were small compared to those reported in the rat. It appears that age-related changes in neuromuscular systems are a function of species and muscle type. The difference between EDL and soleus muscle type (fast and slow, respectively) or to the different usage of the two muscles.

Supported by NIH grant AG-00795

The purpose of the present study was to compare age differences in the amplitude and frequency of miniature endplate potentials in the senescent (0-6 months old) and EDL strains. These animals did not show any deficit of spontaneous or sensory activity related to senescence. However, there was a marked reduction in responsivity to amphetamine, a catecholaminergic stimulant drug, and to dextinidide, a specific anticholinergic drug, with respect to locomotor stimulation. In contrast, the responsivity to cotrimoxazole, a cholinergic antagonist, was enhanced with respect to locomotor depression. Senescent mice also showed enhanced lethality due to cotrimoxazole. Because the changes in drug responsivity were not all in one direction, they are not likely to be due to pharmacokinetic factors. The observed changes in the amphetamine action suggest alterations of cholinergic systems in the senescence. Previously a reduction in the number of choline and acetylcholine binding sites has been shown in the senescent animal. Enhanced activity of a cholinergic agonist and reduced sensitivity to a cholinergic antagonist is difficult to interpret but is probably due to hyposensitivity of the central cholinergic functions.

Supported by NIH Grants Ag 00341 to P.L. and Ag 00538 to G.L. and a grant from the Lederle American Cyanamid Corp.

The hippocampus is one of the major convergent multimodal sensory centers in the brain. The CA1 region of the hippocampus is the site of the acquisition, storage and retrieval of multimodal information designated as short-term memory. One of the more prominent behavioral manifestations of aging is an impairment of short-term memory. In the above observations, it appears likely that age-related structural changes in the hippocampus, especially those having a bearing on neuronal connectivity, may have a direct relationship to the short-term memory impairments observed in the elderly. The purpose of the present study was to compare age differences in the amplitude and frequency of synaptic junctions, axon preterminals and astroglial processes in the stratum radiatum of the CA1 zone in young adult as compared with aged rhesus monkeys on the premise that the data may be extrapolated to the human situation with much greater confidence than in the case of similar studies in rodents.

Quantitative analysis of electron micrographs was performed in two young (approximately 6-8 years of age) and three old (approximately 20 years of age) rhesus monkeys. Fifty random electron micrographs at a magnification of 250,000 times were prepared from each animal. Micrographs were quantitatively analyzed for numerical and volume density (random hit method) for synaptic junctions, axon preterminal and astroglial processes. The numerical density of synaptic junctions and axon preterminals was found to be decreased by 25% and 41%, respectively, in aged animals. In a similar manner, the decrease in volume density of these parameters was 33% and 22%, respectively, of young animals. Synaptic junctions and axon preterminals, astroglial processes demonstrated a marked age-related increase in numerical density (225%) and in volume density (276%). These results confirm and support similar findings which have been reported in the dentate gyrus of the rat and in the human cerebral cortex during aging. This study also demonstrates that marked age-related ultrastuctural alterations occur in the hippocampus, one of the primary convergent multimodal sensory centers in the brain concerned with the mediation of short-term memory.

Supported by NIH RR-00164-16 and by a grant from the Lederle American Cyanamid Corp.

20 LONG-TERM ADRENALECTOMY REDUCES SOME MORPHOLOGICAL CORRELATES OF BRAIN AGING. P.W. Landfield*, C. Murr*, J.D. Lindsay and G. Lynch*. (SPON: Department of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103; 2nd. Dept. Psychobiol., Univ. Calif., Irvine, CA 92717; 3rd. Dept. Neurosci., UCSD Sch. Med., La Jolla, CA 92037). In previous reports we noted that the hypothyroidism of hippocampal astrocytes is a consistent correlate of brain aging in Fischer rats (Landfield et al., 77, Proc. Soc. Neurosci., Abstracts, 78). To test the hypothesis that endocrine systems, in particular the adrenocortical hormones (Landfield et al., 77, Science; Soc. Neurosci. Abstracts, 78). To test the above hypothesis, we conducted a similar experiment in which aging was induced by hypothyroidism and a reduced dose of glucocorticoids. The primary endocrine system was the adrenocortical system. The animals were divided into two groups: normal and adrenalectomized. The normal group received 250 µg/ml replacement glucocorticoid in the drinking saline. A control group was adrenalectomized at the same age and maintained in the same manner. However, these control animals received 250 µg/ml cortisol in the drinking saline (high dose). This group was, however, adjusted on several occasions because of weight loss and some deaths in the high dose group. Final dose was 175 µg/ml. An intact control group was also included. Plasma glucocorticoids were elevated after adrenalectomy and analyzed after the period of steady state. Analysis of semithin brain sections appears to indicate that the low dose animals exhibit reduced numbers of hippocampal microgria and a reduced number of astrocytes. Microgliosis and increased depth are correlates of aging in the hippocampus of rats (Landfield et al., Proc. Soc. Neurosci., 1978; D'Amour et al., Brain Res., 1975; Lindsay and D'Amour, 1979). Lipofuscin appears unaffected by these treatments. Astrocyte analyses are still underway, as are more complete analyses of other variables. To this point, however, adrenalectomy can reduce at least some correlates of brain aging in rats. Of course, whether these reductions are relevant to functional changes still remains to be determined.

Supported by NIH Grants Ag 00341 to P.L. and Ag 00538 to G.L. We thank Dr. Ann Etgen, Matt Maxwell, and Dr. Wayne Simpson for important technical assistance.

A single low dose (0.2-0.35 mg/kg) of d-amphetamine sulfate reinstated lever-presses (1-p) for brain stimulation reward (BSR) in rats no longer 1-p for BSR. These animals ranged in age from 400-550 days, but were implanted at 200-250 days with bipolar platinum electrodes in the medial forebrain bundle in regions of the lateral hypothalamus. All showed vigorous 1-p after implantation and subsequently received drugs, but were drug-free at least 60 days prior to amphetamine injection. Food deprivation, increased stimulating current, frequent priming stimulation and resuming failed to reinstate stable 1-p. Amphetamine injected 5 minutes prior to session reinstated 1-p in 7 of 11 rats with minimal priming and the remaining animals after one shaping session. BSR parameters threshold current and mean resistance to the stimulating current increased over initial values while rate of 1-p remained unchanged. All animals exhibited 1-p on two subsequent sessions without additional amphetamine injection. Three animals continued to 1-p without further injection on five subsequent trials over a 1 month period. These data suggest that amphetamine may rejuvenate degenerating reward systems in aged animals.

(Supported in part by USPHS grants NIH RRO7179-01 and HD 02176-01.)


If an animal becomes ill following the ingestion of a novel substance, the intake of that substance will be suppressed on subsequent presentations. This phenomenon is referred to as conditioned taste aversion (CTA). In an earlier study we found that young and old rats did not differ in their ability to acquire a CTA to saccharin (SAC) when averted with amphetamine. However, retention of the CTA was significantly impaired in the old animal. We also found that this deficit could not be attributed to changes in peripheral mechanisms (i.e., taste, illness, etc.), suggesting that age-dependent changes within the brain and/or pituitary may underlie the impaired performance of the aged rat on this task.

The effects of a variety of stressful experiences, such as CTA, have been shown to be mediated in part by changes in pituitary hormone secretion. In particular, vasopressin has been shown to restore the avoidance response in studies using young rats. Since there is ample evidence suggesting decreased synthesis and availability of vasopressin in the old rat, we investigated the possibility that treatment with exogenous vasopressin (LVP) may improve the performance of aged rats on the retention phase of the CTA.

Male rats 3, 6, 10 and 19 months of age were conditioned to avoid a 0.1% SAC solution by three pairings of the novel taste with a 0.5 mg/kg, ip injection of d-amphetamine. All animals were compared for acquisition and retention of a taste aversion with a two bottle choice process. Animals in all age groups tested acquired the aversion to SAC equally over a period of 2 months. The older animals treated with LVP (1ug/kg) every 5 days retained the CTA significantly longer than did the saline injected controls. The data suggest that the pituitary-adrenal axis may be involved in age-related behavioral deficits in fear-mediated responding.

ELECTROPHYSIOLOGICAL AND BIOCHEMICAL EVIDENCE FOR AGE-RELATED ALTERATIONS IN HIPPOCAMPAL CHOLINERGIC FUNCTIONING. A. S. Lipina, D. J. Critchett, B. T. Bartus (SPON: C. Latimer), Medical Research Division, American Cyanamid, Pearl River, NY 10965; and, W. Harrington and R. W. Pelham, Tufts-New England Medical Center, Dept. Psychiatry, Boston, MA 02111.

While central cholinergic (ACH) mechanisms have recently been implicated in the cognitive disorders associated with old age, no direct evidence for age-related functional alterations in ACH mechanisms has been reported. Since hippocampus has also been implicated in memory functions, we have investigated muscarinic ACH receptors in dorsal hippocampus in male, albino rats aged either 6-9 months (YOUNG) or 26-29 months (OLD). All rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and allowed to breathe spontaneously. Microiontophoresis and recording were performed with stereotaxically placed 5-barrelled glass micropipettes of approximately 6 µm overall tip diameter. The center recording barrel was filled with 1.5 M NaCl and generally had a resistance of 3-12 Megohms. Three of the four side barrels were filled with either ACh (0.5 M, pH 3.4) or L-glutamic acid (GLU, 1 M, pH 8.5) which were passed as cation and anion, respectively. The fourth barrel contained 1 M NaCl to balance drug ejection and holding currents. Dorsal hippocampal pyramidal cells were tentatively identified by current threshold and bursting firing pattern. At the end of each electrophysiological recording session, brains were removed and stored frozen for the subsequent measurement of [3H]-quinclidinyl benzilate ([3H-QNB]) binding and choline acetylase activity. Both ACH and GLU stimulated the firing of pyramidal cells in a dose-related manner (1, 2, 5 and 10 nA). However, the responses to ACH were significantly reduced (p < .004, t test) in the number of binding sites (YOUNG Bmax = 151 ± 5 fmol/mg protein; OLD Bmax = 1330 ± 40 fmol/mg protein) with no significant change (p > .05) in affinity (YOUNG KD = .274 ± .016 nM; OLD KD = .294 ± .035 nM). Choline acetylase activity, a measure of ACH neurons, was unchanged. Since the responses to iontophoretically applied GLU were dose-related, but not age-related, the present results document a selective age-related functional impairment of hippocampal ACH mechanisms.


In a blind, coded study, the hippocampus and frontal cortex from 22 rhesus monkeys ranging in age from 4 to at least 20 years old were evaluated for age-related morphologic alterations at both the light and electron microscopic levels. Based on these findings, we have categorized the rhesus monkey's chronological age as either (I) Yes, (II) Transitional, or (III) Aged. In general, both the light and electron microscopic findings were positively correlated with each other and with the various age groups. The light microscopic evaluation, which was based largely on the degree of senile (neuritic) plaque formation and amyloid deposition was, on the whole, a reliable indicator of age, particularly for those monkeys at either end of the chronological spectrum. EM appeared to provide a satisfactory evaluation for all age ranges, and was particularly effective in detecting those subtle morphological age-related changes in tissue from "Transitional" stage II subjects such as individual or small groupings of abnormal neurites which had not yet formed recognizable plaques. The major EM findings that were associated with advancing age were: an increase in lipofuscin deposit in both neurons and glia, accumulation of glial fibrillary content, enhanced myelin remodeling, and the presence of corpora amylacea and dystrophic and degenerating neurites. Hippocampal changes were usually, but not invariably, more pronounced than the neo-cortical findings.

(Supported by NIH Grant NS-02255)

Previous published (Prog. Neuro-Psychopharmac. 2:107-115, 1978) and unpublished work of the senior author has demonstrated instability of a brain protein called 12.7 in a significant number of schizophrenic and related pathologies. These previous studies were primarily on chronic, aged individuals. The hypothesis tested in this current study states: The instability of protein 2.7 is correlated with age rather than schizophrenia specifically. Groups of schizophrenic, old diseased (non-schizophrenic), old normals, young diseased, and young normals were compared. Although our sample sizes are small for many of the above categories yielding statistically non-significant data, the trends are consistent indicating the possible validity of our hypothesis.

Supported in part by grants from RESEARCH CORPORATION and the NICAMIN FAMILY FOUNDATION to Gary D. Miner, Ph.D.

26 AGING OF CIRCADIAN RHYTHMS IN FEMALE RATS. Sarah S. Mosko and Robert Y. Moore. Dept. Neurosciences, UCSD, La Jolla, CA 92093

During the normal course of aging in female rats, estrous cycling breaks down near the end of the first year and rats enter a constant vaginal estrous (CVE) state at 12-15 mo which is succeeded by a series of repetitive pseudopregnancies beginning at about 2 yrs. The primary deficit responsible for the breakdown in cycling is unknown. Rats with neonatal or adult lesions of the suprachiasmatic nucleus, which contain the circadian pacemaker, exhibit a permanent CVE syndrome. The estrous cycle of rodents has a circadian organization which is timed by the light-dark cycle, and recent evidence indicates that there may exist a circadian signal for LH release which is responsible for the pre-ovulatory LH surge. We compared circadian rhythms in 7 old constant estrous (OCE; 16-18 mo) and 4 old repetitive pseudopregnant (OPRP; 24-26 mo) rats to rhythms in 6 young cycling (YC; 3-4 mo) rats to determine if aging of circadian rhythm generating mechanisms could be a factor in the breakdown of estrous cycling in aging females. In addition, since neonatal androgenization induces a permanent CVE state which has been viewed as a model of early reproductive senescence, we also examined circadian rhythmicity in young, early androgenized (YEA; 3-4 mo) rats (100ug testosterone propionate, s.c., on the day after birth).

Circadian rhythms in locomotor activity and drinking behavior were monitored in all rats (Long Evans, hooded) in both entrained (L:D, 14:10) and free-running (constant dim illumination, 1.0 lx) conditions. The results indicate that circadian rhythmicity in both behaviors declines with increasing age. In diurnal lighting, YC, OCE and OPRP rats restrict 72.7 ± 2.4%, 67.1 ± 1.7% and 56.9 ± 2.2%, respectively, of their drinking behavior to the 10 hrs of darkness. This percentage decrease in aged rats is probably the result of fewer drinking events during the dark phase. Power spectral analysis of drinking events in both entrained and free-running conditions reveal a progressive flattening of the spectrum and a diminished circadian peak with advanced age. YEA rats, in contrast, exhibit normal rhythmicity in activity and drinking.

Our findings indicate that the amplitude of circadian function declines significantly with advancing age in rats. Aging of central circadian rhythm generating mechanisms is implicated. Damaged circadian functions in old age could contribute to the normal pattern of reproductive senescence observed in female rats.

The observation that YEA rats exhibit normal circadian rhythms may indicate that different mechanisms underlie the circadian states of the YEA rat and rat with suprachiasmatic nucleus ablation.

27 PROTEIN SYNTHESIS IN ORGAN CULTURES OF RAT BRAIN MICROVESSELS. Michael A. Hooker*, Jillian Gane*, Brenda L. Cowart*, Monica J. Williams*, Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, MIT, Cambridge, MA 02139.

Biochemical studies have shown that cerebral blood vessels exhibit age-related changes in their protein content which may be important in the development of vascular lesions. To examine the extent to which these changes reflect differences in protein synthesis, we have established an organ culture system capable of supporting protein synthesis in brain microvessels.

Intraparenchymal brain microvessels consisting of arterioles, capillaries, and venules were isolated from the forebrains of Sprague Dawley rats by isometric sucrose density gradient centrifugation and microliter techniques. The microvessel preparations were free of contaminating neurons and glia, as shown by light and electron microscopy and by the absence of radioactivity within microvessels prepared after the addition of radiolabeled synaptosomes and cytoplasmic proteins to the whole brain homogenates. We also examined the protein patterns of these blood vessels by isoelectric focusing and by using dodecyl sulfate polyacrylamide gel electrophoresis, and found that they differ considerably from those of synaptosomes and brain homogenates. The isolated microvessels were incubated at 37°C in the presence of Eagle's minimal essential medium (devoid of methionine) and 35S-methionine under 95% O2 / 5% CO2 conditions. The incorporation of radiolabeled methionine into trichloroacetic acid-precipitable protein appeared proportional to the concentration of vascular protein at ranges of 1-2 µg/ml. The microvessels incorporated methionine at a linear rate up to at least 30 minutes. The newly synthesized proteins, resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and by isoelectric focusing, exhibited some differences between animals of 4 and 21 months. Such studies appear useful as models for examining the biochemical activity of cerebral blood vessels during aging and other pathologic states.


The gill withdrawal reflex to gill stimulation and its neural correlates are age-dependent. The reflex and the physiology and anatomy of L7 and R2, central neurons in the parietovisceral ganglion, were examined in old Aplysia, 500 gm and heavier, and in mature Aplysia, 100-250 gm. No less than 50 days of age separated the two groups. The reflex in old Aplysia was elicited by a stimulus of 1.5 gm, whereas in mature animals 0.2 gm was threshold. Habituation of the reflex, to 1.5 gm, was twice as fast in old Aplysia (n=7) as in mature animals (n=7). L7 staining did not differ between old and mature alypsia but size differences did not appear as L7, 90% of the old were 0.7 M in the old group as compared to 0.48 M in the mature group; decreased Rin can explain reduced PSP size. Time constant, T, in old animals was twice that in mature animals. Size of L7 in living ganglia from the two groups were not significantly different. Rin, T, and size comparisons of R2 were similar to those found for L7. LM and EM studies of L7 and R2 revealed that with aging the number of glial cells increased, and there was increased membrane infolding probably accounting for decreased Rin and greater T. Age-pigment, lipofuscin, in close proximity to the infoldings appeared to be a factor. In addition, the size of age and exhibited autofluorescence similar to that seen in vertebrate neurons (Brizee et al., 1969). In old Aplysia, EM showed that lipofuscin granules were composed of vesicles, vacuoles and lamellated structures. The bodies attained sizes of about 5 µm length. In mature Aplysia and those 60 days young, lamellated structures, and were 0.5 - 1 M. Behavioral and physiological signs of aging relate closely to the anatomical signs. The Aplysia nervous system is appropriate to study the history of individual neurons, esp. those mediating behavior (NIMH; Sanders-Brown Ctr., U.K.)
AGE RELATED CHANGES IN RAT CEREBELLMUM: PURKINJE CELL DENDRITIC DYING BACK, NEUROFIBRILLARY TANGLES, AND LEARNING AND RETENTION IMPAIRMENT FOLLOWING ALUMINUM ADMINISTRATION.

DENDRITIC DYING BACK, NEUROFIBRILLARY TANGLES, AND LEARNING AND RETENTION IMPAIRMENT FOLLOWING ALUMINUM ADMINISTRATION: IMPLICATION FOR BRAIN AGING.


The age-related changes in the cerebellum may play a critical role in the etiology of the disease. To further determine the possible role of aluminum in that disease, it is important to systematically discern the consequences of increased brain aluminum content. Development of a method is described for recording under halothane anesthesia. Several penetrations of the cerebellum, sensorimotor cortex, and dentritic morphology analyzed by the use of the Scholl method. Aluminum treated animals were deficient in both climbing fiber doublets and triplets. Occasionally these cells had dendritic branches with increasing distance from the cell body that matched those of the 28 mos cells, however, had significantly lower mean discharge rates, longer modal ISIs, and greater numbers of long ISIs. In the face of this diminished activity, the rest were highly aberrant with little activity other than climbing fiber evoked bursting. Overall, the 3,10, and 20 mos cells appeared to be more normal patterns, usually for a few seconds within 2-3 min recording epochs. By the use of the Scholl method, the number of long ISIs, the presence of neurofibrillary tangles, and the ultrastructural correlates of aging (e.g., lipofuscin) using electron microscopic methods. Electron microscopic analysis indicated the presence of neurofibrillary tangles in the soma and dendrites of neurons of the cerebellum, hypothalamus, midbrain, and degeneration after topical alumina cream application. Such degenerative changes may represent the resulting course of the dendritic changes observed here, and may be homologous to that observed in brains from the senile human.

Effects of chronic hypoxia on levels of catecholamines in brain regions from aging rats. Isaac F. Knouf, Larry L. Embree and David W. Jackson.

Male Sprague-Dawley rats at early senescence (21-22 months old) were exposed to a mixture of 10% oxygen - 90% nitrogen in a sealed plastic cage for 36 hr. The gas mixture was passed through the cage at a rate of 3.5 l/min. Simultaneously, control animals of identical age received air at the same rate. At the end of this period, the rats were decapitated, the brain excised, and immediately dissected into the following regions: cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, cerebellum, pons and medulla. Spectrofluorometric determinations of norepinephrine (NE) in cerebral cortex, hypothalamus, midbrain, cerebellum, pons and medulla, and of dopamine (DA) and serotonin (5-HT) in these regions did not differ significantly from controls. Gross observation of the brains after 13, 19, and 35 hr showed that the animals in the hypoxic environment were less active as compared with the controls, and the hypothalamic feeding centers were maximally active. The levels of these monoamines in the regions examined did not differ significantly from controls.

Male CBA/J mice have 25 to 50% fewer substantia nigra neurons than male BALB/cJ mice. (Supported in part by NIH grants AM-19761, NS-00259, AG-00847, AG-01656 and NSF grant BNS 78-11153.)

Losses of nigral neurons are a possible mechanism involved in nigrostriatal aging and are undoubtedly involved in Parkinson’s disease. Genetic differences in the subclinical population of dopamine neuron number, coupled with an age-related loss of nigral neurons has been suggested as a mechanism for the age-related onset of Parkinsonian symptoms. We studied the CBA/J mouse as having a “genetic nigrostriatal lesion” through which we could examine the effect of early losses of nigral neurons on catecholaminergic function, particularly on dopamine receptors.

Male CBA/J and C57BL/6J mice were used for genetic differences in the subclinical population of dopamine neuron number, coupled with an age-related loss of nigral neurons. These data indicate that monoamine levels are depressed in the striatum of aged mice. These findings suggest that there may be an age-related decline in dopamine receptor density in the striatum. This may have implications for the development of age-related diseases such as Parkinson’s disease. However, it is not known whether this effect is due to an age-related decrease in dopamine receptors or to a loss of dopamine receptors containing cells.


Recent reports have indicated a number of pre- and postsynaptic alterations in neurodegenerative disorders. Of the various neurotransmitters, the brain cholinergic system appears to be particularly vulnerable with cholinergic changes reportedly occurring in Huntington’s, Parkinson’s and Alzheimer’s diseases. In the present investigation, pre- and postsynaptic cholinergic markers were studied in the brains of aged mice. In aged rats, it was found that the loss of cholinergic markers was due to a decrease in receptor density. It is not known whether this effect is due to an age-related decrease in cholinergic receptor density or binding sites or a loss of cholinergic receptors containing cells.


Human aging is accompanied by alterations in a number of central functions which either are mediated or influenced by monoaminergic neurons; these include locomotion, sleep-wakefulness, mood and others. Synthetic and degradative monoamine enzymes are known to fluctuate with age in a direction consistent with an hypothesis of depressed monoamine levels in the elderly. However, the degree to which this might occur at the single cell level and the location where the effect might be affected is unknown.

Mouse neurons (M. nemestrina); 3 each at 4, 10, 20 years old (y.o.) were prepared from formaldehyde-induced histo-fluorescence. Quantitative microspectrofluorometric analysis of the norepinephrine fluorescence was performed on individual neurons of select, monoaminergic, cell groups and substantia nigra (SN), nucleus raphe dorsalis (Rd). The relative intensities were recorded, means and standard deviations were calculated, and histograms were plotted as histograms. Intensity levels were highest in the 4 y.o., and declined progressively in the 10 y.o. and 20 y.o. Norepinephrine fluorescence intensity was depressed further in neurons of the LC which also contained autofluorescent pigment granules. Monoamine content as reflected in relative fluorescence intensity was depressed as much as 75% in R and SN, and 50% in LC. These data indicate that monoamine levels are depressed in perikarya of origin of major ansergic systems. It is not known whether this is reflected also in the terminal fields supplied by these neurons; our preliminary analysis in these animals suggests that terminal fields in brain stem and hypothalamus are comparable to the 4 y.o., but quantitative NSF was not performed. The present findings of depressed perikarya stores might be related to decreased synthetic capabilities and/or heightened turnover of central monoamines during aging and may correlate with altered functional capabilities.

Supported by NIH grants AM-19761, NS-00259, AG-00847, AG-01656 and NSF grant BNS 78-11153.
OPIOATE RECEPTOR SUPERSENSITIVITY IN SOME BRAIN REGIONS OF AGED MALE RATS. Beatriz J. Vasquez, Rita B. Messing, Robert A. Jensen, Joe L. Martinez, Vina R. Spiehler*, Fernando Samaniego*, and James L. McGaugh. Dept. of Psychobiology, University of California, Irvine, CA 92717, USA.

Previous work from our laboratory indicates that memory changes observed in aged rats may be mediated by endogenous opioid systems. Therefore, we compared dihydromorphine binding kinetics in five brain regions of young and aged F344 male rats. In order to characterize binding in the presence or absence of endogenous substances half of the tissue homogenate for each region was twice washed and resuspended in 0.05M tris buffer, pH 7.4. Aliquots from whole homogenates and washed membrane samples were then used for assays of stereospecific \(^3\)H-dihydromorphine (DHM) binding with one of seven concentrations of the ligand (0.13 to 13 nM). The apparent dissociation constant (\(K_D\)) and receptor density (\(B_{max}\)) were calculated from Scatchard plots.

<table>
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<th>Brain Region</th>
<th>(K_D) (nM)</th>
<th>(B_{max}) (fmol/mg prot.)</th>
<th>(K_D) (nM)</th>
<th>(B_{max}) (fmol/mg prot.)</th>
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*p < 0.05. **p < 0.01 different from young.

Increased affinity for DHM accompanied by decreased receptor concentrations were observed in whole homogenates of frontal poles, amygdala, and striatum of aged as compared to young rats. In washed membranes, however, no difference between young and old rats was apparent in striatal tissue. The results suggest that receptor supersensitivity observed in brain regions of old rats may compensate for decreases in receptor number. Further, these receptor alterations may be related to in vivo differences in the effects of opiates in aged rats. [Supported by USPHS AG00538 and MH12525, NSF BNS 76-17370, and a grant from the McKnight Foundation (all to JLMcG)].


In a number of pathological disorders, the neurofilaments are tightly packed within the cellular processes of brain cells. Our hypothesis was that aging could cause similar effects. In order to test this hypothesis, we cultured brain explants from 24 month old rats and compared outgrowth, 100X filament protein (neurofilament protein) characteristics and general cytoplasmic morphology between aged explants and brain explants from much younger animals, viz., three days and three months of age. All rats were killed by decapitation and cerebral cortex was excised under sterile conditions. Explants were mechanically dissociated on coverslips which were flooded with CMRL 1066, containing 10% horse serum and 5% fetal calf serum. After 7 days, the coverslips were checked for outgrowth of cells. We found that cellular outgrowth is possible although the time of cell spreading may be prolonged. After 2 weeks in culture, the coverslips were fixed with rabbit-anti-bovine neurofilament antibody and fluoresced with FITC goat-anti-rabbit globulin at a dilution of 1:6. Neurofilament fraction for antibody preparation was isolated from bovine spinal cord white matter and characterized by a molecular weight of 51,000. The cytological differences included an increase in the number of lipofuscin granules in cells from aged brains as well as accumulations of vacuoles which were bounded by neurofilaments. Even though cells from aged brains appeared to contain less neurofilament, that which was present was tightly packed and in tortuous bundles both in the cell periphery and surrounding the nucleus; whereas, young brain cells, especially from neonate explants, contained a filamentous network of thread-like neurofilaments in the periphery when compared by immunofluorescence.

Supported by USPHSHL20, HL0042.
AUDITION
39 CYTOLOGY OF PERIOLIVARY CELLS. Joe C. Adams. LNO, NINCDS, NIH

Bethesda, Md. 20205.

Clusters of auditory cells located around the superior olivary complex are referred to as periolivary groups rather than nuclei because of their lack of well defined borders or neuronal cell bodies. These cells are arranged in rows with respect to their apical and basolateral orientations and dendritic fields. As an initial step towards a comprehensive analysis of these neuronal groups, we have studied the periolivary complex in the cat and the hamster, using techniques employed in the isolation and culture of the medial olivary complex. These cells exhibit regular resting discharge and a high mean firing rate. Some seventeen classes of cells were identified with respect to their size, shape, and Nissl substance. These cells are concentrated close to the caudal surface of the lateral superior olive. Cells of this type extend rostrally for a short distance dorsal to the lateral olivary complex. Ventral to the lateral olivary complex extend rostrally for almost its entire extent. Pale cells are found caudally, predominantly dorso-medially to the medial olivary complex. Ventral to the medial trapezoid nucleus many cells have only a few large, dark clumps of Nissl substance. Many cells in the lateral trapezoid region have only a few small Nissl clumps. Concentrations of large pale cells are found caudally in the rostral region of the sensory macula, while each unit with regular resting discharge innervate large numbers of hair cells in the cranial nerve medial to the intact otic capsule. After functionally: E.R. Lewis.

40 NET-ENKEPHALIN POSITIVITY IN THE SMALL CELLS OF THE DEEP DORSAL COCHLEAR NUCLEUS AND POSTEROVERTEBRAL COCHLEAR NUCLEUS OF THE RAT. Richard A. Altschuler. Laboratory of NeuroOtolaryngology, NIH, NINCDS, Bethesda, Md. 20205.

Clusters of auditory cells located around the superior olivary complex in the cat and the hamster, using techniques employed in the isolation and culture of the medial olivary complex. These cells exhibit regular resting discharge and a high mean firing rate. Some seventeen classes of cells were identified with respect to their size, shape, and Nissl substance. Neural connections have been shown to exist between the cochlear nuclei and the superior olivary complex. These connections may be mediates via the ventral cochlear nucleus. Fibers were also seen in the posterior and anterovertral cochlear nucleus. These connections may be mediates via the ventral cochlear nucleus. Fibers were also seen in the posterior and anterovertral cochlear nucleus.

In conclusion, net-enkephalin containing cell bodies were localized in both the dorsal and posterovertral cochlear nucleus. In both cases these neurons were mainly concentrated near the olivary complex. Some seventeen classes of cells were identified in the hamster cochlear nucleus near the border of the dorsal cochlear nucleus. These neurons were also categorized as small cells.

Positive fibers were shown to exist between the cochlear nucleus and the olivary complex. Neural connections have been shown to exist between the cochlear nuclei and the superior olivary complex. These connections may be mediates via the ventral cochlear nucleus. Fibers were also seen in the posterior and anterovertral cochlear nucleus. These connections may be mediates via the ventral cochlear nucleus. Fibers were also seen in the posterior and anterovertral cochlear nucleus.

The laminar nucleus is found caudally just ventral to the CN. As it extends rostrally in the anterior and posterior branches of the bullfrog VIIIth cranial nerve medial to the intact otic capsule. After functionally. V. E. Lewis.

41 CORRESPONDENCES BETWEEN STRUCTURE AND FUNCTION IN THE BULLFROG UTRICLE AND LAGENA. R. Bojke. Dept. EECG, UC Berkeley, CA 94720.

Previous studies have shown that the utricle and lagena are innervated by afferent axons whose diameters range from 1 to 10 μm (Honn, A.), J. Comp. Neurology. 182: 621-636, 1978), that these axons can be divided functionally into several classes based on regularity of resting discharge and response to constant-velocity tilt (Blanks, R. and Precht, W. J. Comp. Physiol. 125: 379-390, 1976), and that the hair cells innervated by these afferents can be divided into several classes based on surface morphology (Lewy, E. and Precht, W. J. Comp. Physiol. 125: 379-390, 1976). It has been proposed that each unit with irregular resting discharge and phasic response to tilt corresponds to a thick afferent originating from a small number of hair cells in the cranial region of the sensory macula. Each unit with regular resting discharge and tonic response to tilt corresponds to a thin afferent originating from a large number of hair cells in the extrastriolar region of the macula (Fernandez, C. et al., J. Neurophysiol. 35: 978-997, 1972). To test this proposition directly, intracellular recordings were made from primary afferents in the anterior and posterior branches of the bullfrog VIIIth cranial nerve medial to the intact otic capsule. After functionally. E.R. Lewis.


The cytoarchitecture in the torus semicircularis (TS) was examined in 14 brains. After perfusion the brains were prepared for cresyl violet staining or Golgi-Kopsch impregnated Alaride-embedded sections. The TS was examined in 14 brains. After perfusion the brains were prepared for cresyl violet staining or Golgi-Kopsch impregnated Alaride-embedded sections. The TS was examined in 14 brains. After perfusion the brains were prepared for cresyl violet staining or Golgi-Kopsch impregnated Alaride-embedded sections. The TS was examined in 14 brains. After perfusion the brains were prepared for cresyl violet staining or Golgi-Kopsch impregnated Alaride-embedded sections.
OBSERVATIONS ON UNIT ACTIVITY IN MONKEY AUDITORY CORTEX AND DORSOLATERAL FRONTAL CORTEX DURING A SOUND LOCALIZATION TASK.

Denis P. A. Benson, Robert D. Hienz*, and Moise H. Goldstein, Jr., Dept. Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD. 21205.

Behavioral studies have suggested that the auditory cortex plays an essential role in the localization of sound in space and that the dorsolateral frontal cortex may be involved in spatial discrimination tasks. In this experiment five small speakers were equivalently spaced from 75° left to 75° right and within arm's reach of a rhesus monkey subject. The animals were trained to locate the source of a 100-msec sound burst by pressing the key on the speaker which presented the sound (localize). In a control condition (detect), animals reported the onset of any sound regardless of location by pressing a single key which was not adjacent to any sound source. For each unit recorded, the sound-evoked activity during detect was compared to the activity evoked by the same stimulus during localize. In a third condition the same stimulus was presented without requiring any response from the animal (non-perform). In four animals approximately 8% of the units recorded from primary auditory cortex and surrounding secondary areas had significantly greater evoked activity (p<.01) during localize than during detect without any change in spontaneous rate or in the type of discharge pattern. Over 20% of the units which had greater evoked activity in the performing conditions (detect and localize) than in non-perform.

Recordings were also made in the dorsal pericalcarine area, a region of frontal cortex which receives input from auditory cortex. Although only a small proportion of frontal units responded to sound during non-perform (40%) or during localize and detect at a latency of less than 100 msec. These units also responded with similar latencies and discharge patterns to visual stimuli presented at the speaker locations, provided that the animal localized the visual stimuli by pressing a key adjacent to the light.

These results suggest that, unlike behavior, the responses of neurons in auditory cortex are dominated by auditory input, whereas sound-responsive neurons in frontal cortex are more likely to share visual input and to be more strongly influenced by the specific behavioral task.

(Supported by NSF Grant No. BNS76-81793)

ACTION OF GLUTAMATE AND RELATED SUBSTANCES ON THE SPONTANEOUS ACTIVITY OF AFFERENT NERVES IN THE TOAD LATERAL LINE.


The purpose of this study was to extend our results which showed glutamate mimics the afferent transmitter released by hair cells in the guinea pig cochlea (Bobbin, Exp. Brain Res., 34:389, 1979) and in the lateral line (Bobbin and Thompson, Assoc. Res. Otolaryngol., Jan. 1979) by examining the actions of related chemicals, other putative transmitters, and their interactions. Spontaneous activity of a single lateral-line stalk from Xenopus laevis was studied using an isolated skin preparation. Ringer's solution was constantly washed over the inner surface of the skin, except for 5 minutes before and 7 minutes during drug testing. Drugs were applied to the end organ by ejection from a microliter syringe. Results indicate that the application of several substances produced excitation, with larger concentrations producing greater excitation followed by inhibition of spontaneous activity. Ranking these according to degree of excitatory potency was as follows: kainic acid (10 μM) > L-glutamate (2 μM) > D-glutamate (2 μM) > D-aspartate (2 μM). Only inhibitory activity was exhibited by DL-aminoadapate, ATP, tyramine, GABA and salicylate. Ranking all substances according to degree of inhibitory potency was as follows: L-glutamate (10 μM) > D-glutamate (2 μM) > D-aspartate (2 μM) > DL-aminoadapate (2 μM) > ATP (2 μM) > tyramine (5 μM) > GABA (5 μM) > salicylate (5 μM). The excitatory action of L-glutamate was blocked when applied to the skin during the inhibition following application of aminoadapate, kainic acid, or L-glutamate. At present, how or where the substances are acting is unknown, although results suggest that kainic acid is the transmitter released by the hair cells. (Supported by NIH, USPHS NS-11647 and NS-07058, and The Kresse Foundation)

EFFECT OF INTRACOCHLEAR KAINIC ACID ON COCHLEAR POTENTIALS IN GUINEA PIGS.


Evidence has been presented which implicates glutamate as a possible transmitter released by hair cells (Bobbin, Exp. Brain Res., 34:389, 1979). To investigate the chemical nature of the synapses between hair cells and primary auditory neurons, we studied the effects of intracochlearly perfused kainic acid (KA) on sound evoked, as well as resting potentials in the cochlea. Experiments were performed on guinea pigs with intracochlear perfusion methods which have been previously described (Bobbin and Thompson, Ann. Otol., 87:185, 1978). Input-output functions for the compound whole nerve action potential (CWAP) and cochlear microphonic potential (CM) were obtained to a 10-kHz tone burst. Results reveal that 1 mM KA abolishes the CWAP but has no effect on CM or the endocochlear potential. Perfusion with Ringer's after the KA produced no identifiable recovery of the CWAP. To further evaluate this effect by KA, experiments have been performed studying the action of intracochlearly perfused 1 mM KA on spontaneous cochlear ganglion cell activity. These experiments indicate that KA produces an initial, excitatory increase in spontaneous discharge rate followed by suppression of activity. Although the mechanism and site-of-action of KA in the cochlea is unknown, the results are consistent with the hypothesis that postsynaptic membranes of the auditory nerve fibers have receptors for glutamate. Possible putative transmitters on hair cells and supporting cells, however, cannot be excluded. These results provide additional, indirect evidence that glutamate may be involved in synaptic transmission between hair cells and first-order afferent auditory neurons. (Supported by NIH, USPHS NS-11647 and NS-07058 and The Kresse Foundation)

DYNAMIC VERSUS STATIC CHARACTERISTICS OF SINGLE AUDITORY-NERVE FIBERS.

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Although the operating range of an auditory-nerve fiber can be defined as the range of intensities over which changes in intensity produce changes in response, the observed operating range depends to some extent on the experimental paradigm. Firing rates averaged over a period of 30 milliseconds to reflect an ineradicably static operating characteristic with a small operating range (Smith, R. L., J. Acoust. Soc. Am. 65, 166-179, 1979). For example, in response to stimuli of constant sound intensity, a maximum firing rate occurs at the onset followed by a decay to a quasi steady-state rate within a few hundred milliseconds. The steady-state firing rate typically increases monotonically with sound intensity, and asymptotically approaches saturation within 30 dB of threshold. After subtraction of spontaneous activity, the ratio of onset to steady-state response is independent of sound intensity so that the onset and steady-state responses have the same operating range. In contrast, when the onset firing rate is measured using intervals of 2 msec or less, the ratio of onset to steady-state response increases with increasing sound intensity. In some units the increase continues into the steady-state saturation region, producing a dynamic operating characteristic with a range that exceeds the static range by more than 20 dB. Amplitude modulation by sinusoids reveals a similar increase in operating range. For modulation frequencies of about 200 Hz, period histograms synchronized to the modulating waveform have a sinusoidal shape and provide a measure of response modulation. Response modulation is a nonmonotonous function of average intensity, and the shape and location of the response modulation function can be more accurately predicted from the dynamic than from the static operating characteristic. Hence the use of sufficiently small time windows and/or sufficiently rapid changes in intensity reveals a dynamic operating range that can exceed the static operating range by several orders of magnitude.

The response characteristics of superior olivary complex (SOC) neurons were observed in unanesthetized decerebrate cats. This report deals only with those units that were excited by best frequency (BF) stimulation to the ipsilateral ear and inhibited by contralateral ear stimulation (EI units). Ninety percent of these units were localized to the lateral superior olives. Spontaneous activity was seen in all EI units and permitted demonstration of ipsilaterally driven inhibitory responses. The presence of inhibitory sidebands was a common feature of the ipsilateral receptive field. The contralateral receptive field was inhibitory and overlapped the ipsilateral central excitatory region, in those units for which it was determined.

Discharge rate could be a monotonically or nonmonotonically increasing function of intensity for ipsilateral BF stimulation. Concomitantly, a monotonically decreasing rate-intensity function as inhibition became more pronounced. Binaural rate-intensity functions became more symmetric. Ipsilaterally driven rate-intensity functions became monotonic if they were already so, and binaural rate-intensity functions displayed no inhibition. Responses measured after administration of anesthetic were similar to those previously reported for SOC units in intact preparations. Quantification of these results will be presented.

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Recent evidence suggests that glutamate and/or aspartate may be the neurotransmitter(s) at acoustic nerve endings in the cochlear nucleus (CN). Godfrey et al. (J. Histochem. Cytochem. 25:417, 1979) have reported that kainic acid, a glutamate analog, when applied iontophoretically to nerve terminals and has also described a calcium dependent release of glutamate onto neurons in the posterior ventral cochlear nucleus (PVCN). This study was undertaken to examine aspects of neuronal systems providing input to the superior olivary complex of the rabbit ventral cochlear nucleus and the superior olivary nucleus (SOO) of the rabbit. The present study was used to examine aspects of neuronal systems providing input to the superior olivary complex of the rabbit ventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN) which can induce changes in the response thresholds of these neurons to tone-burst stimulation at best frequency and after administration of anesthetic doses of sodium pentobarbital. In the few EI units for which we carried out this procedure in the most dramatic case, the almost total disappearance of spontaneous activity. Anesthetic also produced an increased response latency. Post-stimulus time histograms showed chopper responses which were not observed in unanesthetized preparations. Interspike interval distributions became more symmetric. Ipsilaterally driven rate-intensity functions became monotonic if they were already so, and binaural rate-intensity functions displayed no inhibition. Responses measured after administration of anesthetic were similar to those previously reported for SOC units in intact preparations. Quantification of these results will be presented.

Auditory sensitivity as a function of frequency has been determined using shock avoidance techniques for several specimens of clown knifefish, Notopterus chitala (Family: Notopteridae). The frequency range of hearing is from 100 Hz to over 1500 Hz, with best sensitivity occurring around 500 Hz, where the threshold reaches ~50 dB re: 1 dyn/cm². Thresholds as low as ~50 dB have been reported for only a few other fish in the teleostean infraclass: several species (including the goldfish, Carassius auratus) all belonging to the superorder Ostariophysi and one other, Myripristis kuntee, from the squirrelfish family Holocentridae. Best threshold data reported here are at least 20-30 dB lower than those reported for most other teleost fishes. While the frequency range of hearing for N. chitala also extends beyond the high frequency range reported for most teleosts (800-1000 Hz), it does not extend as high as those reported for M. kuntee (3000 Hz) or for ostariophysan species (2000-5000 Hz). Fish from these three taxonomically unrelated groups, Notopterus, Myripristis, and Ostariophysii, all have in common a non-homologous specialization of the peripheral auditory system: an anatomical connection between the swimbladder and the inner ear. Since for many teleost species the swimbladder has a range of hearing for N. chitala also extends beyond the high frequency end reported for most teleosts (800-1000 Hz), it does not extend as high as those reported for M. kuntee (3000 Hz) or for ostariophysan species (2000-5000 Hz).

52 IONIC BASIS OF THE RECEPTOR CURRENT IN A VERTEBRATE HAIR CELL. D. P. Corey* and A. J. Hudspeth, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The unusually high K⁺ concentration of the endolymph facing the apical surfaces of virtually all vertebrate hair cells suggests that K⁺ carries the receptor current in these cells (Russell and Sellick {1976}, J. Physiol. 252: 245). Yet large intracellular receptor potentials can be recorded in frog macular hair cells when a standard saline solution bathes both apical and basal cell surfaces, and thus when the electrochemical gradient for K⁺ is near zero (Hudspeth and Corey {1972}, Proc. Nat. Acad. Sci. U.S.A. 70: 2407). Calcium has also been proposed as carrying the receptor current (Sand {1975}, J. Comp. Physiol. 102:27), yet its concentration is extremely low in the endolymph of some species, including the bullfrog.

We have measured the voltage dependence of the receptor current in frog macular hair cells by voltage-clamping single cells with two microelectrodes, while mechanically stimulating individual hair bundles. The reversal potential for the receptor current, with standard saline on both cell surfaces, is ~ 2 ± 3 mV. This suggests that the transducer channel permits both Na⁺ influx and K⁺ efflux: it is relatively nonspecific. In vivo, with endolymph on the apical cell surface, K⁺ must, in fact, carry most of the receptor current.

A number of monovalent cations were subsequently tested with an in vitro microphonic preparation (Corey and Hudspeth {1979}, Biophys. J. 26: 499), in which the fluid facing the apical hair cell surface may be changed independently of that facing the basal surface. The microphonic potential is maintained when endolymph in the apical chamber is replaced with salines containing 130 mM Li⁺, K⁺, Rb⁺, Cs⁺, or NH₄⁺. The response to noise is reduced with tetraethylammonium or tetrabutylammonium, and is small but measurable with gluanosine, triethylammonium, or tris(hydroxymethyl)aminomethane ion. If the channel is a pore, the largest of these ions would require a minimal pore diameter of ~3Å. The current recorded measured under voltage-clamp varies non-linearly with voltage, and the current-voltage relation can be adequately fitted by a single exponential energy barrier, placed approximately 40Å from the pore mouth.


An equal parts mixture of L-[3H] proline and L-[3H] fucose (50 μCi/μl) was injected into a transverse duct of the labyrinth of each of two White King pigeons. A total amount of 1 μl of the above solution was injected through a micropipette glued in to a transsected anterior semicircular duct over a period of 1 hr. Following injection, the cut ends of the ducts were sealed by cautery and the animals were allowed to survive 15 days. The brain, spinal cord, and labyrinth were fixed with 10% buffered formalin delivered by bilateral transcardiac intracardiac catterization. Serial paraffin sections (15 μm) were prepared by standard autoradiographic techniques.

Auditory structures, ipsilateral to the side of injection, which were labeled were the: cochlear nerve, angular nucleus, magnocellular nucleus, superior olive, lateral lemniscus, laminar nucleus, and ventral nucleus of the lateral lemniscus. Labeled structures, contralateral to the side of injection, included the: laminar nucleus, superior olive, lateral lemniscus, and ventral nucleus of the lateral lemniscus.

Vestibular structures, ipsilateral to the side of injection, which were heavily labeled were the: vestibular nerve, all six vestibular nuclei, and the cerebelo-vestibular lateral process. Less heavily labeled ipsilateral structures included: the medial longitudinal fasciculus, terminations on motoneurons in the medulla, a lateral part of the ventral gray of the spinal cord and the abducens, oculomotor, and trochlear nucleus. Contralateral vestibular structures which were heavily labeled were: the medial longitudinal fasciculus, a terminations on the medial and lateral part of the ventral gray of the spinal cord, and the oculomotor, trochlear, and abducens nucleus.

Different sites of labeling within the oculo- motor nucleus. The lateral half of the dorsal part and the majority of the ventral part of the contralateral oculomotor nucleus showed heavier labeling: whereas, the dorsal part of the ipsilateral oculomotor nucleus was labeled the least.

Structures which were also labeled bilaterally were: the qinto-frontal tract, the septomesencephalic tract, and a region in the ventro-medial part of the primitive paleostriatum. (This work was supported in part by grants from the Geof. Res. Fdn., NASA, NAS9-14641, and NIH, NS-12481.)


Most studies of fish hearing seek to explain behavioral performance only in terms of sound detection by the otolithic maculae of the ear. These are presumably the primary sound detectors in many species, but they may not be the only ones. Many authors overlook the potential contribution of the macula neglecta, a non-otolithic sensory complex found almost universally in fishes. In its most characteristic form this macula is a transverse array of two patches of sensory epithelium that contain oppositely polarized populations of hair cells. A gelatinous cupula lies over these hair cells and in cases where there is acoustic coupling to the external medium the cupula presumably causes hair cell stimulation by being well matched to the acoustic impedance of the aqueous sound transmitting medium that surrounds the fish (Fig. A). In contrast the hair cells of the otolithic maculae are covered by dense masses believed to act as inertial elements poorly matched to the impedance of the surrounding medium (Fig. B).

In the present study unit and population responses in nerve VIII have been evoked by acoustic stimulation of relatively intact fish. The initial population response to a click is composed of a series of brief high amplitude peaks lasting 2.0-4.5 ms. Certain peaks are believed to originate at the macula neglecta, as they are reversibly altered by covering the fenestra ovalis, a sound portal important in macula neglecta stimulation. Other peaks appear unaffected at otolithic maculae, as they are unaffected by this manipulation. The response characteristics and the anatomical distribution of units within the VIIIth nerve also suggest origins of different detection channels. These channels may be active in audition by sharks, and as a result of their dissimilar modes of stimulation they may provide different types of information concerning the nature of acoustic stimuli.

(Supported by grants to Dr. T.H. Bullock from NSF and NIH.)
RESPONSES TO THE DIFFERENCE TONE IN THE ANTEROVENTRAL COCHLEAR NUCLEUS OF THE CAT. John Dickson, Robert Wickersham* and Mary Morton Glosen, Dept. of Neurophysiology, Univ. of Wisconsin, Madison, Wisconsin, 53706.

We have studied the responses of anteroventral cochlear nucleus neurons to the difference tone, \( f_2 - f_1 \). Single cell activity was recorded extracellularly in vivo and anesthetized cats. The lower primary frequency, \( f_1 \), was varied stepwise from slightly above the response area to 20 kHz or more. The higher primary, \( f_2 \), was chosen to maintain \( f_2 - f_1 \) equal to the neuron's characteristic frequency. We measured discharge rate and strength of phase locking to the difference tone at three to six stimulus levels for each pair of primary frequencies. The sound pressure level of \( f_1 \) was always equal to that of \( f_2 \).

With the difference tone frequency and the levels of the two primaries held constant, one might expect that the discharge rate would be independent of the primary frequencies. This, however, is not the case: the response varies with primary frequency, sometimes from a negligible response to saturation. Enhancement and diminution of the response could be accounted for by production of the primaries of other combination tones such as \( 2f_1 - f_2, 3f_1 - 2f_2, \) etc. Thus enhancement could result when a combination tone falls within the response area of the neuron, while reduction could result when it falls within the suppression region of the combination tones on \( f_1 \). In some cases there is an overall decrease in responsiveness at higher primary frequencies could be altogether accounted for by interference with higher order distortion products. In some cases there is an overall decrease in responsiveness at higher primary frequencies could be altogether accounted for by interference with higher order distortion products.

Auditory Pathway. Essential auditory structures, such as the cochlear duct and basilar membrane are first evident in reptiles. Since reptiles are also endowed with the ability to hear, it is reasonable to assume that the auditory system in this Class has proven to be of increasing significance in the understanding of the hearing mechanism.

The present study represents a preliminary report of the characteization of auditory nuclei located in the auditory tubercle (rostral end of the posterior root of the trigeminal nerve) of the red-eared turtle (Chrysemys scripta elegans). At the IM level with cresyl violet or toluidine blue staining, the most easily identifiable cells are large (19-23 um) pale staining "clear" cells with eccentric nuclei and prominent nucleolus. These cells may be representative of the nucleus magnocellularis and are located between bundles of axons. At the IM level many of the auditory tubercle in close proximity to the ependymal layer. Although other types of cells are present, in this report our attention is directed to the large "clear" cells and surrounding neuropil.

The cytoplasm of "clear" cells has a diffuse Nissl substance, the rough endoplasmic reticulum appearing in single, isolated rows. Mitochondria are small (570 nm) and birefringent and are distributed throughout the cytoplasm; numerous lysosomes (900 nm) are usually present. The Golgi complex is present characteristically around the cell center. The cytoplasm is multinucleated; the nuclei are located in the central region, the cell shape is a slightly flattened oval with a few processes.

In order to compare the avian ototoxic response to the mammalian, we injected kanamycin sulfate into the yolk sac of White Leghorn embryos on the 7th day (stage 31) of the 21 day incubation period. Endolymphatic damages were observed by light microscopy and histochemistry. In addition, the medium concentration of mitochondria within dendritic terminals, endolymphatic clusters are also present within dendritic spines. The supporng synaptic arrangements are characterized by an array of various organelles, which have been previously described in a number of species. The majority of sampled synaptic morphology indicates a range of means for axonal transmission. Most of the terminals contain large dense-cored vesicles (1300Å), in addition to clear vesicles and vacuoles. The synaptic vesicles are usually described as being filled with a single neurotransmitter: the intercellular material disperses. Depending upon whether their terminals are mildly or severely damaged, afferent nerves either blow out or explode; afferent nerves, which form synaptic contacts several days later than efferent, show negligible evidence of intoxication. Throughout intoxicated supporting cells, circular zones of clear cytoplasm that are usually bounded by mitochondria or dense borders press the empty holes. In the basal region, the endolymphatic reticula (ER) are irregularly arranged and do not form the normal pattern seen above the superior. In intoxicated cells, the Golgi complexes lose their concentric arrangement and disperse. Kanamycin, which apparently intoxicates along a gradient, damages terminal cells above the inferior fibrocartilaginous plate more severely than those above the superior. Tegmental dark cells leave a mosaic of large spaces that as they withdraw their tangle of snugly fitting cytoplasmic processes; in light cells the sequence proceeds similarly but less conspicuously because of their limited processal number. In intoxicated cells of both types, levels of glycogen increase markedly; in intoxicated individual cells, the Golgi complexes lose their concentric arrangement and disperse. Kanamycin, which apparently intoxicates along a gradient, damages terminal cells above the inferior fibrocartilaginous plate more severely than those above the superior. Tegmental dark cells leave a mosaic of large spaces that as they withdraw their tangle of snugly fitting cytoplasmic processes; in light cells the sequence proceeds similarly but less conspicuously because of their limited processal number. In intoxicated cells of both types, levels of glycogen increase markedly; in intoxicated individual cells, the Golgi complexes lose their concentric arrangement and disperse.

Prenatal Otoxicity of Kanamycin in the Chick. C. D. Fermin and C. N. Cohen. Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

Recently several investigators showed that kanamycin exerts similar otoxic actions during intrauterine life as in the adult. In order to compare the neuropathology in the chick, we injected kanamycin sulfate into the yolk sac of White Leghorn embryos on the 7th day (stage 31) of the 21 day incubation period. Endolymphatic damages were observed by light microscopy and histochemistry. In addition, the medium concentration of mitochondria within dendritic terminals, endolymphatic clusters are also present within dendritic spines. The supporng synaptic arrangements are characterized by an array of various organelles, which have been previously described in a number of species. The majority of sampled synaptic morphology indicates a range of means for axonal transmission. Most of the terminals contain large dense-cored vesicles (1300Å), in addition to clear vesicles and vacuoles. 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AUDITION


To date, no studies have compared the response properties of auditory units with the parameters of communicatory sounds produced by any fish. Because of the large body of descriptive and experimental studies on sound production in the toadfish Opsanus tau, it is an ideal species for such a study. Single units were isolated extracellularly from the anteroventral cochlear nucleus (AVCN). The map was constructed to localize auditory nerve fibers to the cochlear nucleus. Based on a myriad of previous microelectrode studies, it is presumed that the binaural-interaction component must be located caudal to the colliculi yet rostral to the cochlear nuclei. These results suggest that although the inferior colliculus is a powerful integration center for the processing of binaural signals, the neural structures necessary for the maintenance of the BIC must be located caudal to the colliculi yet rostral to the cochlear nuclei. Based on a myriad of previous microelectrode binaural-interaction studies, the nuclei of the auditory cortex are the target regions for the remaining binaural interaction. In addition, wave 3, which shows no binaural interaction, was also affected by the lesion (Fig. 1). This wave reflects activity from a region such as the medial nucleus of the trapezoid body which receives virtually only contralateral monaural inputs.

60 EFFECTS OF MIDLINE BRAIN STEM LESIONS ON THE SHORT-LATENCY AUDITORY EVOKED RESPONSES. B.C. Fullerton and H-L. Hosford*, Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114.

Short-latency (<8 msec) auditory evoked responses (AER) to clicks were recorded with a vertex electrode referenced to one ear canal in 10 adult cats anesthetized with Dial. Ten per second clicks were presented first monaurally to each ear and then binaurally. Recordings were amplified, filtered (1.5Hz-8kHz), and averaged during an intensity series of 5 dB steps up to 60 dB above threshold. An example of a monaural response is shown by the solid line in Fig. 1. We assessed the binaural interaction in the AER by subtracting the binaural (B) responses from the sum of the monaural responses (L+R) to obtain a binaural difference trace (BD), seen in Fig. 2. We found little difference between B and L+R through the time of wave 3. After wave 3, B was generally smaller than L+R with the greatest binaural difference appearing during waves 4 and 5. Binaural interaction apparently occurs for these later waves, but not for the earlier waves.

We examined the acute effects of midline lesions on the binaural interaction seen in the AER. Six cats received lesions intended to sever the ascending fibers to the contralateral auditory cortex. The greater the involvement of the ventral trapezoid body, the greater the effect on the BD. In one case with a lesion severing the fibers of all 3 tracts, the remaining binaural interaction was negligible (Fig. 2), except for high stimulus levels when acoustic cross-talk becomes significant. The waves showing a binaural interaction are significantly affected by this lesion because they almost certainly involve activity from binaurally innervated brain stem regions. In addition, wave 3, which shows no binaural interaction, was also affected by the lesion (Fig. 1). This wave may reflect activity from a region such as the medial nucleus of the trapezoid body which receives virtually only contralateral monaural inputs.


A lesion technique under visual control was used to clarify the possible sites of origin of the binaural-interaction component of the brainstem auditory evoked response in guinea pigs (BIC-BSER). The BIC is obtained by summing BSERs elicited by monaural clicks to each ear and subtracting this sum from the BSER elicited by binaural clicks. Typically, the BIC waveform is biphasic (negative - positive). At stimulus intensities of 20 dB SPL, latency values are 3.5-4.0 ms and 4.5-5.0 ms, respectively (Dobie and Berlin, Arch. Otolaryngol, in press and Proc. Natl. Acad. Sci., USA, 1980).

The response properties of a unit do not depend on its BF. Maximal spike rate and slope of spike-rate curves increase and latency to tone bursts decreases with increasing frequency regardless of a unit's BF. There is no BF. The greatest at BF. At BF, units are tonically active and single-unit data indicate the fish is quite capable of hearing it. This mismatch may enable toadfish to respond preferentially to the relatively loud boatwhistle of nearby callers, since it is difficult to localize distant sounds underwater.

62 RELATIONSHIP BETWEEN TONE DURATION AND THRESHOLD FOR NEURONS IN THE ANTEROVENTRAL COCHLEAR NUCLEUS OF THE CAT. Herz, Morton Gibson, John M. Dickson, Robert E. Wickersberg, Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin, 53706.

It is well known from human psychoacoustic observations that the threshold for detection of a tone is lowered when the duration of the tone burst is increased. This change in threshold is generally thought to be due to temporal integration in higher centers. However, previous physiological experiments suggest that the dependence of threshold on tone duration is detectable in the activity of anteroventral cochlear nucleus neurons and seems to result from the probabilistic nature of the discharges of auditory nerve fibers.

The responses of single cells with low spontaneous firing rates were recorded extracellularly in the anteroventral cochlear nucleus of barbiturate anesthetized cats. The stimulus generally consisted of 500 msec, best-frequency tone bursts presented once per second. The sound pressure level (SPL) was varied in the normal, intermediate, and quiet-ear conditions. The STI is 40 dB at 1 meter, and its fundamental frequency varies from under 150 Hz to over 250 Hz, depending on temperature and assumed environmental state. Even though the toadfish has no neurons primarily tuned to the boatwhistle, behavioral and single-unit data indicate the fish is quite capable of hearing it. This mismatch may enable toadfish to respond preferentially to the relatively loud boatwhistle of nearby callers, since it is difficult to localize distant sounds underwater.
Intracellular and extracellular potentials in the organ of Corti of the Mongolian gerbil. 

David A. Goodman* and Robert L. Smith (SPON: J. J. Zwislocki). Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Class microelectrodes were inserted through scala tympani into the organ of Corti of anesthetized Mongolian gerbils and aimed toward the inner hair cells. The resting and sound-evoked potentials encountered were consistent with those reported by Ruse and Sellick (J. Physiol. 284, 261-290, 1978) in the guinea pig with respect to resting potentials and response magnitude, polarity, and frequency-selectivity. As in their studies, two classes of cells were encountered. Cells contacted first in a penetration, presumably supporting cells, had large resting potentials of -70 to -100 mV. In these cells the depolarizations in response to sound (summing potentials) were less than 3 mV. The second class, presumably inner hair cells, had smaller resting potentials of -15 to -45 mV and large summating potentials reaching 60 to 90 mV. The sound-evoked potential increased as the electrode advanced and reached up to 3 mV in the vicinity of the inner hair cells where it exhibited a frequency-selective component comparable to that of the fibers. With further small advances of the electrode, scala media was entered as evidenced by the positive endolymphatic potential and a polarity reversal of the summating potential. The magnitude of the summating potential measured in the extracellular space near a supporting cell was generally smaller than that measured in the cell. A possible explanation for this inequality is that the summating potential spreads passively through electrically coupled supporting cells with a space constant exceeding that of the extracellular space.
MATERNAL RETRIEVAL TO KITTEN STRESS CALL

We have studied the relation between the ipsilateral projection patterns from field A and the binaural organization of AI. In some experiments each physiologically defined suppression column coincided with a region of dense labeling and each physiologically defined suppression column coincided with a region of sparse labeling. This is exactly opposite to the pattern formed by terminal calls which interconnect the primary auditory cortex in the cat (Hinman, 1977). However, sparse labeling was seen in other experiments in which suppression columns still appeared to be the major recipients of the projection but in addition dense projections were also seen. Variation was also seen in the pattern of projection from field P upon AI. Injections into the same isofrequency strip produced dense elongated bands of label in the center of AI in some experiments while in others labeling was confined to regions near the dorsal and ventral borders. This variability in labeling suggests that neurons which differ in location along an isofrequency strip in field A or P may also differ in their pattern of projection upon field AI. (NSF 76-1983, HD-03552)


The effects of a period of unilateral input attenuation on the responses of central auditory neurons to binaural stimulation have been examined. Input attenuation was produced by ligation of the external auditory meatus in 13 neonatal (12-48 hr) and 5 adult cats. After survival periods of 80-100 days the ligature was removed under barbiturate anesthesia and the status of the peripheral auditory system was assessed by auditory-evoked action potential audiometry. In those animals with normal peripheral sensitivity, the responses to binaural stimulation of neurons in the central nucleus of the inferior colliculus contralateral to the previously-ligated ear were examined by conventional extracellular recording techniques. Observations were restricted to that class of neuron commonly sensitive to interaural intensity difference (IID) in normal cats, viz. those receiving predominantly excitatory input from the contralateral ear and inhibitory input from the ipsilateral ear. Detailed quantitative observations have been made on 32 neurons in neonatally-ligated animals and 24 neurons in adult-ligated animals. Excitatory input from the contralateral (previously-ligated) ear, as reflected in threshold and discharge rate at best frequency, did not differ significantly from that in normal cats. However, ipsilateral inhibition, as reflected in the steepness of IID functions, was considerably reduced in both neonatally- and adult-ligated animals. This reduction in inhibition was particularly apparent in the sustained component of the discharge pattern.

These preliminary results suggest that the auditory system is characterized by a form of plasticity quite different from that observed in the visual system following the analogous procedure of monocular eyelid suture. It appears that binaural components of the auditory system, confronted with attenuated input from one ear, somehow reduce the input from the other (normal) ear so as to maintain a balance of input from the two sides. Such a mechanism would have the consequence that important binaural functions - notably localization - would not be totally impaired by unilateral input attenuation. This "balance of input" hypothesis provides an explanation of the clinical observation that humans with one ear plugged are impaired in an isofrequency strip while a normal or equal-intensity input to the opposite ear.

The previous work in rabbit has shown the composition of the 2-D-oxo-D-glucose (2-DG) technique can be applied to studies of the auditory pathway (Jones and Dieterhoft, '79). The present work in rabbit is part of an examination of the pattern of metabolic activity which may be present during tone or noise stimulation. This work was carried out in the 3rd and 4th weeks post weaning age. The auditory structures were stimulated with pure tone pulses, and the deoxyglucose uptake and autoradiographs were made on X-ray film. Cresyl violet stains were also used to verify the results obtained from the autoradiographs. The distribution of labeled deoxyglucose in the brain was determined by autoradiography following stimulation of the auditory system. The distribution of labeled deoxyglucose in the brain was determined by autoradiography following stimulation of the auditory system. The distribution of labeled deoxyglucose in the brain was determined by autoradiography following stimulation of the auditory system. The distribution of labeled deoxyglucose in the brain was determined by autoradiography following stimulation of the auditory system.


The boundaries of the dorsal nucleus of the lateral lemniscus (DNLL) were studied in the dorsoventral plane of cats. The dorsal DNLL was marked by a combination of the Golgi-impregnated neurons and their orientations in Nissl sections showed a nearly cubical nucleus with maximal dimensions of 1.1 mm (dorsomedial) × 1.2 mm (ventromedial). The DNLL nuclei were clustered with individual clusters separated by thick fascicles of lemniscal axons. The width (W) of the DNLL was determined at 0.64 μm in cats. The dorsal DNLL was enclosed by the thalamus, the hypothalamus, and the reticular thalamic nucleus. The DNLL was demonstrated with the Golgi-impregnated neurons and their orientations in Nissl sections showed a nearly cubical nucleus with maximal dimensions of 1.1 mm (dorsomedial) × 1.2 mm (ventromedial). The DNLL nuclei were clustered with individual clusters separated by thick fascicles of lemniscal axons. The width (W) of the DNLL was determined at 0.64 μm in cats. The dorsal DNLL was enclosed by the thalamus, the hypothalamus, and the reticular thalamic nucleus.
76 EAR OCCLUSION CAUSES SYSTEMATIC SHIFTS IN THE RECEPTIVE FIELDS OF AUDITORY UNITS. Eric L. Knudsen and Masakazu Konishi, Div. of Biology, Calif. Inst. of Technology, Pasadena, Calif. 91125.

The ear openings of the barn owl (Tyto alba) are asymmetrically located on the head: the left ear is higher than the right. A consequence of this asymmetry is that the left ear is more sensitive to areas of space below the horizontal plane and the right ear is more sensitive above. Behavioral experiments show that ear occlusion causes systematic errors in the owl's ability to localize sounds: when the left ear is plugged, the owl localizes above and to the right of the sound target; when the right ear is plugged, it localizes below and to the left of the target.

A specialized region in the midbrain (MLD) of the owl has been implicated in spatial analysis of sound. Units in this region only respond to sounds from restricted areas of space (receptive fields) and are inhibited by sounds originating outside these areas. Furthermore, the units are arranged within this brain region according to the locations of their receptive fields so that they form a physiological map of auditory space.

We used a movable sound source to map the receptive fields of these units both before and after plugging one ear. All units were shifted in the direction opposite to that caused by ear plugging. We used a movable sound source to map the receptive fields of these units both before and after plugging one ear. All units were shifted in the direction opposite to that caused by ear plugging.

The following effects due to varying stimulus off time and/or interaural intensity have been observed in these neurons. 1) As stimulus off time was decreased, the degree of modulation of the frequency cycling could markedly increase. This effect can be relatively independent of the stimulus duration. 2) As the stimulus off time was increased, the symmetry of the interaural delay curve could be altered considerably. For example, at longer off times the cleft behavior of the interaural delay curve was abolished when the stimulus to the contralateral ear was delayed, but was preserved when the stimulus to the ipsilateral ear was delayed. 3) These asymmetrical effects due to stimulus off time could sometimes be offset and/or reversed by varying interaural intensity. Neurons which exhibited one or more of these properties were similar in that the neural response to a nonaural stimulus was of an onset type with a latency of at least 0.2 ms.

Such manipulations are helpful in understanding the time course and interactions of the activity generated by each ear. For example, the effect of stimulus of time on the degree of modulation of the cycling can be understood in terms of a time dependent inhibition. These results suggest that some inferior colliculus neurons may be sensitive to particular stimulus attributes.

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76 CYCLIC RESPONSE PROPERTIES OF CAT INFERIOR COLICLUS NEURONS AS A FUNCTION OF INTERAURAL DELAY: EFFECTS OF TIME AND INTENSITY. S. Kuwada*, T.C.T. Yin, and R.E. Wickesberg (SPON: D. Oertel). Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, Wis. 53706.

When a low frequency sinusoid (~3000 Hz) is presented dichotically, the response of many inferior colliculus neurons will vary cyclically as a function of the interaural delay. The phase shift between peaks of the interaural delay curve corresponds to the period of the stimulus cycling frequency. The present study describes a small number of neurons in the inferior colliculus of the cat with unusual response properties: the cycling nature of their interaural delay curves was altered as a function of stimulus off time and/or interaural intensity.

Cats were anesthetized with sodium pentobarbital and single cell activity was monitored extracellularly with platinum-gold plated indium microelectrodes. A computer was employed for controlled stimulus delivery, data collection and subsequent off-line data analysis.


Numerous studies have established beyond question the existence of functional asymmetries between the two cerebral hemispheres in man. Dichotic listening proved to be a powerful tool for investigating such asymmetries. This technique has often been used to study hemispheric specialization in normals and commissurotomized subjects. However, very few studies have been conducted in patients suffering from callosal agenesis using the dichotic paradigm. Two subjects were included in the experimental group: MC, age 8 and LG age 16 had a complete callosal agenesis as revealed by CT scan. The patients were matched with two control groups: a. subjects with normal IQ, b. subjects with an IQ comparable to the acallosal patients (~75). Each was right handed and had bilaterally normal hearing at the speech frequencies. All subjects were tested in an auditory recognition task under dichotical listening and their performances (mean errors and reaction time) were compared. Verbal (common words and nonsense syllables) and non verbal (pure tones) stimuli were binaurally presented to the subjects through earphones. Results have shown that 1) in the normal and matched IQ groups, the right hemisphere was superior when performing tasks whereas when listening to a verbal stimulus the left hemisphere performed better with the verbal stimuli 2) in the acallosal patients, the right hemisphere is always superior independently of the nature of the stimulus presented. However, the reaction times recorded from the acallosal patients are three times longer than the other groups. These results tend to show that acallosal patients acquire during their development a brain asymmetry different from normal.
BRAINSTEM AUDITORY EVOKED POTENTIALS AND AUDITORY RADIATION ACTIVITY IN THE MONKEY. Alan D. Legatt, Joseph Arezzo* and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Short latency or brainstem auditory evoked potentials (BAEP), recorded during the first few msec following a click stimulus, are believed to reflect activity in the brainstem auditory pathway. The BAEP waveform recorded in the monkey resembles that seen in man, although some differences can be identified. We have examined the relationship between evoked activity in the geniculocortical radiations and the far-field BAEP in the monkey. We used 95 DBS clicks and found that the activity mainly peaked at a rate of 2 to 10 per second on an alert monkey which had been implanted with arrays of vertically-oriented guide tubes for depth electrodes. Depth passes were made through contralateral auditory cortex and into the underlying white matter. Three-dimensional field mapping delineated primary auditory cortex on the basis of potential gradients, polarity inversions, and multiple unit activity (MUA) maxima.

The same components were identifiable in the depth recordings in and around primary auditory cortex as in the simultaneously-recorded BAEPs at the vertex up through wave 7, which had a peak latency of 3.7-4.0 msec. An additional positivity, peaking at 4.2-4.4 msec and larger than wave 7, was seen in these depth recordings; it was not seen as a distinct component at the vertex.

Wave 8 at the vertex peaked at 4.9-5.3 msec. The corresponding depth-recorded potential was much larger; it could be traced upwards from auditory cortex into its far field. It did not invert in polarity beneath auditory cortex, as did the cortical AEP. MUA in the deeper layers of primary auditory cortex showed two bursts preceding the cortical BAEP, peaking at 5.4-5.6 msec.

No depth pass penetrating the auditory radiations recorded a N1A burst, peaking at 5-6.0 msec, restricted to a distance of approximately 0.5 mm. Its positive potential correlated with a negative-positive-positiverelative positive wave onset at 4.5 msec and peaks at 5.5, 6.0, and 6.2 msec. The initial positivity could be traced upwards, its latency gradually approaching that of wave 8. A small N1A burst with an onset at 4.3 msec and a peak at 4.5 msec was recorded in the middle, but not below, the N1A resistance.

Thus it appears that wave 8 at the surface to a large degree reflects propagating action potentials in the geniculocortical radiations. The activity recorded at the middle latency N1A burst and additional positivity in the depth recordings involves only a small part of the radiations and cortical projection areas, and is seen as an individual wave at the scalp.

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Recent electrophysiological data from single auditory-nerve fibers suggest that three unit types can be differentiated on the basis of thresholds and rates of spontaneous discharges (Liberman, J. acoust. Soc. Amer. 43:442, 1979). A recent study by Yano et al. (J. acoust. Soc. Amer. 64: s85, 1978) proposed a hypothesis that some high-frequency units originate at the basilar papilla. In order to obtain direct evidence on this matter, intracellular recordings were made from primary afferents of the frog amphibian papilla. These afferents were penetrated in the posterior branch of the VIIth cranial nerve medial to its emergence from the internal auditory meatus. After characterization of the frequency response properties of each unit, we attempted to fill the axon by iontophoretic injection of Lucifer Yellow (W. M. Stewart, who provided the Lucifer Yellow used in this research.

Field potential depth profiles are relatively independent of the electrode placement over the DCN. This suggests that there is no significant net lateral current flow resulting from eighth nerve stimulation, a condition necessary to perform a one-dimensional CSD analysis. CSD calculations reveal a current sink in the region of the fusiform cell basal dendrites and a current source of approximately equal magnitude in the DL. We believe that this source-sink distribution reflects primarily the dendritic currents of fusiform cells.

Excitability cycle studies of the field potential in upper DL revealed no significant effects of conditioning stimuli on the test response at intervals between 10 and 200 msec, suggesting that a monosynaptic or very secure disynaptic pathway is responsible for the evoked potential. One possible disynaptic pathway originates in the anterior ventral cochlear nucleus (AVCN). In one cat, the DCN source-sink distribution and time course in response to eighth nerve shock were not modified by transection of the dorsal intranuclear association fibers from the CN. Impulse activity recorded in the dorsal cochlear nucleus in response to noise or branch of the VIIIth cranial nerve medial to its emergence from the internal auditory meatus. After characterization of the frequency response properties of each unit, we attempted to fill the axon by iontophoretic injection of Lucifer Yellow (W. M. Stewart, who provided the Lucifer Yellow used in this research.

Physiological studies have shown that the eighth nerve terminals on the basal dendrites of fusiform cells. If we assume that the eighth nerve terminal is a current sink in the region of the fusiform cell basal dendrites reflects activation of eighth nerve terminals. The current source in the DL would then be expected passive current flow through the apical dendrites of the fusiform cells. The observed distribution of current sources and sinks is in full accord with the known topological distribution of eighth nerve synapses on fusiform cells.

Previous work (L. E. Marks, J. Acoust. Soc. Am. 64: 107, 1978) showed that the two ears exhibit linear binaural summation of the loudness of pure tones: Total loudness equaled the sum of the loudnesses of the left-ear and right-ear components. The present series of six experiments used the method of magnitude estimation to measure binaural summation of stimuli with various spectra, presented at equal and unequal intensity to the two ears. Narrow bands of noise (one-quarter octave at 1000 Hz) behaved like pure tones, giving linear summation of loudness in sones. (Loudness in sones is proportional to the 0.6 power of sound pressure.) Wide bands (300-4800 Hz) of white noise (flat spectrum) and pink noise (spectrum declining 3 dB/octave) showed less than complete summation of loudness in sones. Two-tone complexes with narrow spacing (860 and 1160 Hz) and wide spacing (300 and 4800 Hz) gave complete summation, like single tones and narrow-band noise, whereas a complex with intermediate spacing (675 and 1475 Hz) gave incomplete summation, more like the summation found in wide-band noise. The results imply an interaction between the process of loudness summation by the two ears and the process of loudness summation across sound frequency.


Apomorphine agonists induce stereotyped motor responses and hyperthermia in rodents. However, these agents may not have the same effects in different rodent species in terms of auditory responses; acoustic startle in rats is enhanced by apomorphine (APO). Latencies of auditory brainstem evoked responses (BSER's) are increased by hyperthermia, which offers a possible explanation for seizure protection. We looked for direct effects of APO on auditory afferents by obtaining BSER's while body temperature was maintained. C57BL/6 mice were anesthetized with pentobarbital and core temperature was maintained at 37.5 - 37.8°C. BSER's were recorded before and after injection. Due to rapid habituation of startle to a 112 dB click stimulus, we used only 5 stimulus presentations before and another 5 after injection. When compared to saline injected controls, APO mice which were hyperthermic had startle response latencies increased by 5.4 msec (p < .01) and response amplitudes reduced by 48% (p < .01). The two APO groups also differed from each other, with the hyperthermic subjects having longer latencies (p < .01) and smaller amplitudes (p < .01). These data are in contrast to those obtained in the rat. However, the APO induced depression of acoustic startle in mice does correlate with the protection from AGS's that APO affords.

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The axonal projections of spiral ganglion neurons which provide the primary afferent innervation to the ipsilateral cochlear nucleus were investigated by the technique of orthograde axonal transport of HRP (Mesulam et al., 1979). We used HRP in information retrieval for experimental studies. Young adult guinea pigs received intracochlear injections of 50% chromatographically-purified HRP (Huntington) dissolved in 5 ml of physiological saline. Following post-injection times of 24 hrs, the animals were perfused with a 24 glutaraldehyde in phosphate-buffered fixative and the brainstems post-fixed for one day in 24 glutaraldehyde in 15% sucrose solution and for an additional day in a 30% sucrose solution. After fixation, the brain was prepared for frozen sectioning and cut at 40 µm. The summated tissues were then reacted with tetramethylbenzidine (TMB, Sigma) following a procedure similar to that described by Mesulam (J. Histochem. Cytochem., 26:106, 1978). Sections were subsequently mounted and stained with neutral red.

Recent studies using intracochlear HRP injections and subsequent histochemical processing with diamobenzidine (Ross et al. Acta Otolaryngol. 84:187, 1977) have suggested that HRP is not actively transported to brainstem auditory nuclei, but rather results from diffusion processes. In contrast, our results using the TMH-reaction procedure appear to indicate that anterograde transport from the cochlea to cochlear nucleus can be demonstrated. Specifically, the morphologically-distinct regions of anteroverentral and postoverentral cochlear nuclei were heavily labelled with reaction product, while only the deep layers of the dorsocochlear nucleus showed dense accumulations of reaction material. These results indicate a specificity similar to that demonstrated by tritiated amino acids known to be actively transported from the cochlea to the cochlear nucleus. Additional evidence in support of these findings will be presented based upon injections of HRP following ototoxic insults to the cochlea.


The lissencephalic cortex of the rabbit provides opportunities for the correlative study of its functional and architectonic features, opportunities that are not available when studying gyrate brains. Using multunit and evoked potential activity, we have studied the auditory cortex of urethane anesthetized rabbits, New Zealand rabbits and guinea pigs. The orientation of the isofrequency contours and the best frequencies of neuron clusters were determined at lowest thresholds, and at highest thresholds. For the rabbits, the isofrequency contours were found to be reproducible and to be well defined. The best frequencies were determined by the number of neurons which had activity responses to pure tones ranging from 0.4 to 35 KHz. Although some neurons were found which had no apparent best frequency, our results indicate that higher frequencies (15-35 KHz) are represented dorsoventrally in the temporal cortex. The electrode tracks were placed so as to pass as much as possible through lamina III and IV. As much as 2% glutaraldehyde in phosphate-buffered fixative and the electrode tracks were histologically verified from Nissl stained sections. In the regions where the AEP was observed earliest, large amplitude negative AEPs predominated. Once within the active zone, the electrode was advanced in 250 µm steps and recorded from the cochlear nucleus. Additional discriminated unit activity and averaged evoked potentials (AEPS) were recorded in response to tone bursts, white noise and clicks monaurally delivered to the contralateral ear. Sound pressure levels were monitored. Electrocorticographic lesions were placed at selected sites and the electrode tracks were histologically verified from Nissl stained sections. Some of the brains have been processed by the Golgi-Cox method to permit subsequent analysis of the spatial projection of the dentate systems (Glasser et al., Exp. Brain Res., 1979, in press). The AEPS have been found to be useful "signposts" of the electrode's location. The AEPS have been observed well before responsive unit activity was encountered. In the regions where the AEPS were observed most consistently, the electrode's initial invasion of the region yielded acoustically-responsive unit activity was accompanied by AEPS that were multiphasic and initially positive. As the electrode advanced through the auditory zone, large amplitude negative AEPS predominated. Once within the active zone, the electrode was advanced in 250 µm steps and the best frequencies of neutron clusters were determined at lowest thresholds. Neurons with the action potentials found to be responsive to tones ranging from 0.4 to 35 KHz. Although some neurons were found which had no apparent best frequency, our results indicate that higher frequencies (15-35 KHz) are represented dorsoventrally in the temporal cortex. These findings provide evidence for the tonotopic organization of rabbit auditory cortex, and are in agreement with the organization of the auditory fields observed by others in squirrel and guinea pigs. The orientation of the isofrequency contours and the possible existence of several auditory fields are currently under investigation. Supported by NSR Grant BNS 78-05502.

Audition
OTOTOXIC ACTIONS OF AMINOGLYCOSIDES IN COCHLEAR PERFUSIONS CORRELATE WITH IN VITRO EFFECTS ON POLYPHOSPHOINOSITIDES. Iris N. Mechelgast, Shahid Lobitz, Norman D. Weisberg, and Jochen Schafer.

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The cochlea of the guinea pig is a suitable organ for the biochemical investigation of hearing because it is readily accessible under preservation of its function. The perilymph-filled spaces surrounding the inner ear tissues lend themselves to the perfusion with solutions containing radiotracers for the labeling of tissue metabolites or with drugs which affect hearing. These fluids are perfused through capillaries implanted into the scala vestibuli and tympani. During these perfusions, sound can be introduced by an earphone seated in the external meatus, and the cochlear microphonic potential (CM) is obtained via an electrode in the inner capillary. In addition to monitoring the integrity of the inner ear, these simultaneous CM measurements allow correlations of biochemical reactions with the physiological state of the cochlea which can be manipulated, e.g. by exposure to ototoxic drugs.

Aminoglycoside antibiotics have specific toxicity against the inner ear and the kidney. The ototoxicity of seven aminoglycoside antibiotics and fragments was measured quantitatively by cochlear perfusion: artificial perilymph was perfused for one half hour allowing a stable CM to be achieved, followed by 10 µM drug in artificial perilymph for one hour. Continuous measurements of CM were made throughout the perfusions. Kanamycin B and neomycin caused the most rapid decline of CM followed by gentamicin C1a, ribostamycin and kanamycin A. Neamine and methyleosaminic did not show significant ototoxicity. We have previously shown that polyphosphoinositides inhibit the turnover of polyphosphoinositides in the inner ear and the kidney in vivo as well as in vitro. Furthermore, these drugs were shown previously to interact directly and specifically with polyphosphoinositides in monomolecular films of these lipids, implicating polyphosphoinositides as possible receptors for the ototoxic drugs. To test this hypothesis, we measured the interactions of the above seven antibiotics with monomolecular films of polyphosphoinositides. Neomycin and kanamycin B induced the largest increases in surface pressure followed by the other drugs in the same order as seen for ototoxicity. The correlation between in vivo and in vitro action of the drugs was r=0.91. These results support the binding to the polyphosphoinositides as an important part in the mechanism of aminoglycoside ototoxicity. Furthermore, an in vitro assay system seems possible for the assessment of this drug toxicity.

(CSponsored by NIH Grant NS-13792 and Program Project Grant 05785)

COCHLEAR NUCLEUS PROJECTIONS TO THE INFERIOR COLLICULUS OF THE CAT STUDIED WITH LIGHT AND ELECTRON MICROSCOPIC AUTORADIOGRAPHY. Andrea L. Geisler and M. Kent Hord.

We have previously shown that polyphosphoinositides inhibit the turnover of polyphosphoinositides in the inner ear and the kidney in vivo as well as in vitro. Furthermore, these drugs were shown previously to interact directly and specifically with polyphosphoinositides in monomolecular films of these lipids, implicating polyphosphoinositides as possible receptors for the ototoxic drugs. To test this hypothesis, we measured the interactions of the above seven antibiotics with monomolecular films of polyphosphoinositides. Neomycin and kanamycin B induced the largest increases in surface pressure followed by the other drugs in the same order as seen for ototoxicity. The correlation between in vivo and in vitro action of the drugs was r=0.91. These results support the binding to the polyphosphoinositides as an important part in the mechanism of aminoglycoside ototoxicity. Furthermore, an in vitro assay system seems possible for the assessment of this drug toxicity.

(Supported by NIH Grant NS-13792 and Program Project Grant 05785)

COCHLEAR NUCLEUS PROJECTIONS TO THE INFERIOR COLLICULUS OF THE CAT STUDIED WITH LIGHT AND ELECTRON MICROSCOPIC AUTORADIOGRAPHY. Douglas L. Oliver and D. Kent Hord.

We have previously shown that polyphosphoinositides inhibit the turnover of polyphosphoinositides in the inner ear and the kidney in vivo as well as in vitro. Furthermore, these drugs were shown previously to interact directly and specifically with polyphosphoinositides in monomolecular films of these lipids, implicating polyphosphoinositides as possible receptors for the ototoxic drugs. To test this hypothesis, we measured the interactions of the above seven antibiotics with monomolecular films of polyphosphoinositides. Neomycin and kanamycin B induced the largest increases in surface pressure followed by the other drugs in the same order as seen for ototoxicity. The correlation between in vivo and in vitro action of the drugs was r=0.91. These results support the binding to the polyphosphoinositides as an important part in the mechanism of aminoglycoside ototoxicity. Furthermore, an in vitro assay system seems possible for the assessment of this drug toxicity.

(Supported by NSF and NIH.)
THE STRUCTURE AND CORtical PROJECTIONS OF THE MEDIAL GENICULATE BODY OF THE RAT. Hugh A. Patterson* and W. Bruce Warr. Department of Anatomy, School of Medicine, UCSF, San Francisco, CA, and Human Communication Research Institute for Communication Disorders in Children, Omaha, NE.

The structure of the rat medial geniculate body (MGB) was studied in photographic reconstructions from normal material stained with Nissl, myelin and reduced silver methods. Largely consistent with previous work in various species, the rat MGB has three principal cell groups, a ventral, medial and dorsal division, here termed the suprageniculate nucleus, was recognized.

The cortical projections of these nuclei were studied by both the HRP and Fink-Heimer methods. All parts of the MGB have cortical projections and these projections complement the structural parcellation just described. The ventral division projects topographically to a core area of koniocortex, area 41 of Krieg. Terminal degeneration was localized primarily in layers IV and III. The medial division projects diffusely to areas 41 and to extensive areas dorsally and anteriorly contiguous to it. This projection appears to involve the ventral layers of cortex, but with heavier concentrations in layer VI. Terminal degeneration was also identified in the suprageniculate nucleus, was recognized.

The activity of single neurons in auditory cortex of rabbit was monitored continuously throughout the acquisition of a classically conditioned nictitating membrane response to an auditory stimulus at the characteristic frequency of each unit. Stimula
tion and recording techniques were previously described (Perkins and Distefano*, '76). All naive rats were conditioned during initial conditioning trials, in which the animal was behaviorally naive, was compared with that occurring during the later trials in which the conditioned response was established. The magnitude of this type of CM is enhanced by a
hypoxic exposure or by hypoxia. This latter type of CM is enhanced by a
hypoxic exposure or by hypoxia. The magnitude of this type of CM is enhanced by an
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conditioning

The study supports previous work in which multiple unit activity was shown to vary systematically with the acquisition of a behavioral response to an auditory stimulus (Buchwald et al., '66; Oleaon et al., '75, Distefano and Stuart, '76). The fact that changes did not occur in all of the units studied, suggests that additional factors may be involved. The variety of response changes observed reflects the heterogeneous response properties characteristic of single neurons in auditory cortex.

Supported by NIH Grant No. 5 ROI NS12137.

Cochlear Microphonic Potentials from Inner and Outer Hair Cells: Martha G. Plesser* and Anne Muller*. (Baton: Maryanna Henkari, Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda MD 20014; Dept. of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213)

Generally it is not considered feasible to distinguish the cochlear microphonics (CM) originating from outer hair cells from that originating from inner hair cells and extracellular recordings. The large number of contributing hair cells, their stimulated phase distribution, and the finding that there is a 40-dB difference in their sensitivities has promoted this notion. In the present study it is reported that within a narrow window of stimulus intensity functions displays a feature suggestive of a dual origin: it has two maxima separated by a cancellation notch at about 70 dB SPL. In addition, the cell's response to selected, repetitive acoustic stimuli was assessed before and after behavioral manipulations. Alterations in unit response to both the CS and UCS were prevalent in conditioned animals. Response plasticity was most frequently expressed as an increase in unit activity, although decreases also occurred. Subcomponents of a response pattern could change independently of each other. New responses, absent prior to conditioning, emerged after training. Shifts in spontaneous rate did not necessarily occur in the same direction as changes in stimulus-evoked activity. In pseudoconditioned animals, response patterns remained predominantly unchanged, or became attenuated over time. Some of the changes described above also occurred, but to a lesser extent, in this group.

The horseshoe bat, Rhinolophus ferrumequinum, identifies and tracks its prey by emitting loud orientation cries and listening to the returning echoes. Each echolocation sound is composed of a constant frequency (CF) component of about 83 kHz lasting for about 10-100 msec followed by a high frequency, modulated part. While hunting, the CF component of the echo will have a higher frequency than the emitted CF due to a Doppler-shifting of the echo caused by the Doppler effect that occurs during the echo. The Doppler-shifted echo is stabilized and actively held at a frequency called the reference frequency. The frequency of the reference echoes is characteristic from each of the bats studied by us were between 83.6-84.1 kHz. By recording the best frequencies (BF) of several hundred neurons it was found that the inferior colliculus is functionally divided into two regions: one dorsal and one ventral. In the dorsal region, extending to a depth of about 800 microns, the BFs increase systematically with depth from 9 kHz to about 77 kHz. In marked contrast, the BFs in the ventral region varies only a constant along a particular dorsoventral axis with neurons having similar BFs being arranged in cortical-like columns. The range of BFs in the ventral region extends from about 78-88 kHz along the anterior-posterior axis with columns tuned to 78 kHz located most anteriorly and columns tuned to 88 kHz being situated most posteriorly. Thus, the neurons in the ventral region are tuned to BFs that span the range of BFs emitted and echo CF components can assume during echolocation.

There also exists a pronounced disproportionate representation of frequencies within the range of 83-84.5 kHz. This small frequency band corresponds closely to the frequencies at which these bats manipulate the echo. This binding of CF to CF suggests that the over-represented frequency region of the brain is analogous to the foveal regions in the visual system. The audiometric window has the similar shape, which is characteristic to the visual system is several respects. Instead of eye movements, these bats manipulate their voices (compensate for Doppler-shifts) in order to stabilize the echo. The auditory system of horseshoe bats appears to be similar to the auditory system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, 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and, in some respects, resembles the visual system of other bats in a similar frequency range and, in some respects, resembles the
Typically, the caudal part of the saccular macula in nonostrogylinus telestes has a group of dorsally oriented hair cells (kinocilium on the dorsal side of the cell) above a group of ventrally oriented cells. The rostral part of the region has posteriorly oriented cells above a group of anteriorly oriented cells. Variants on this basic pattern are found in each osteoglossomorph except Ostrogylinus. Pantodon has alternating anteriorly and posteriorly oriented cells on the rostral macula region. Notostomus is unique in having only vertically oriented cells on the saccular macula. This pattern has heretofore only been known for the saccular maculae in Osteichthyes and terrestrial vertebrates. The saccular pattern in Notostomus also differs from that seen in previous studies of Ostrogylinus. The saccular macula is essentially tripartate, and thin 'bridges' connect each of the three regions. The most anterior macular region contains a group of anteriorly oriented cells at the rostral tip, followed by a continuation of the anteriorly oriented cells ventro-lateral to a posteriorly oriented cell group. The middle part of the macula contains a group of ventrally oriented cells rostral to a group of dorsally oriented cells while the third posteriorly oriented cells above ventrally oriented cells.

The lagena macula in Ostrogylinus, Pantodon, and Notostomus is morphologically similar to nonostrogylinus telestes in having a dorsally oriented group of hair cells located anterior to a group of ventrally oriented cells. However, the macula in Notostomus more nearly resembles the pattern in Ostrogylinus and has hair cells oriented in a wide range of directions.

The functional and taxonomic significance of these observations are not yet clear, particularly with regard to the nomenclature. It is possible that the diversity in saccular ultrastructure is indicative of there being striking differences in the way that each of these species detects or processes acoustic information. (Supported by NSF and by an RCDA from NINCDS.)


Recent studies have shown that several lower auditory system structures are effected by barbiturate anesthetics. Use of the unanesthetized, decerebrate preparation has revealed differences in spontaneous rates, in inhibitory response areas and firing patterns, as computed to the anesthetized, intact animal.

We have studied the posteroventral cochlear nucleus (PVCN), within the cochlear nucleus complex (CNC) of the anaesthetized cat. The PVCN consists of two major regions; an anterior multilayered cell area (AMCA) and a posterior octopus cell area (POCA). The AMCA is believed to receive most of their eighth nerve input on the dendrites. A chopper response is most frequently recorded in this region of the anesthetized cat. The AMCA is made up almost entirely of octopus cells, which receive massive eighth nerve input onto their somas and proximal dendrites. It has been reported that octopus cells receive two different types of synapses from the same eighth nerve fiber. Singleunit recordings in the AMCA of anesthetized cats have displayed onset responses to tone bursts, and little or no spontaneous activity.

Within the AMCA of decerebrate cats, the most frequently recorded response is the chopper pattern, with less than 5 msec. at between peaks. This response seems to be independent of the units spontaneous rate and interspike interval distribution. Chopping intervals greater than 5 msec. have not been observed.

Within the AMCA of the unanesthetized cat, the spontaneous rates range from 0 to 135, with a mean of 34 spikes/sec. Approximately 80% of the responses recorded from the AMCA are of an onset type. One response consists of an onset, followed by a profound inhibition for the remainder of the tone burst. A second response type consists of an onset, followed by a low level of excitation for the remainder of the tone burst. These units have tuning curves that are generally broader than those from eighth nerve fibers. These data will be compared to other findings from the auditory system of decerebrate cats. (Supported by USPHS Grants NS12209 and 1MO13020.)


Eighteen day old C57Bl/6J mice exposed to noise for 90-seconds at 110 dB SPL (bandwidth, 8-73 kHz). At 23 days a bipolar concentric electrode was placed in the cochlear nucleus (CN) of anesthetized mice. Neurons of CN were then isolated with a micropipette and the CN was then explored in animals exposed to the noise and in non-noise exposed control subjects. First, tone-burst evoked response threshold sensitivity was measured in 5 test frequencies from 5 to 39.0 kHz. Next, simultaneous two-tone masking procedure was introduced to define evoked-response tuning curves at each of 6 central frequencies. The CN was then explored, and in noise exposed animals the threshold sensitivity in the noise exposed animals was between 12 and 20 dB for test frequencies from 10.0 to 26.0 kHz. At other frequencies no threshold loss was noted. The frequency selectivity changes in threshold sensitivity and frequency selectivity, and suggest that the effects of noise in the present experiment may be damage confined to the outer hair cells of the organ of Corti. The C57Bl/6J mouse strain exhibits a critical period of heightened susceptibility to acoustic trauma at 15 days of age and the present data contribute to our understanding of this phenomena. (This work was supported by an award from the Deafness Research Foundation.)

Preparations were obtained from male South African clawed frogs (Xenopus laevis, Duttaphrynus melanostictus). Tissue was perfused with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.12M sodium phosphate buffer, pH 7.2, and processed for autoradiography with standard methods (Schoen & Sokoloff, 1979).

Technical details are given in full in the Methods section of the paper. The main points are as follows: 1. ANAS was originally detected by the Tulane University School of Medicine, New Orleans, La. 70112. 2. The biodetection preparation responds regularly to the presence of ANAS. Bull frog ANAS was originally used for this purpose, but it has since been replaced by ANAS of other species, such as guinea pigs and rats. 3. The biodetection preparation consists of single axons of the bullfrog saccule-saccular nerve. Only the last mentioned axons are observed in the ventral CN and the intermediate acoustic stria, with less intense labelling in the dorsal CN. 4. ANAS produces intense labelling of terminals in the mouse ventral CN which had a similar distribution to that of primary auditory nerve terminals. 5. Electron microscopic autoradiography is underway to identify the labelled structures.

Supported by support from Grants ME 9823 & 14593.


A new auditory projection field with unique functional characteristics has been located within the inferior colliculus using the 1-2-deoxyglucose metabolic mapping technique (Science, 187:447, '75). This field consists of a band of high active elements located at the ventromedial extremity of the central nucleus of the inferior colliculus. In barbiturate anesthetized preparations receiving no acoustic stimulation, this projection area (VM) is seen autoradiographically labelled bands of 1-2DG in width that rings the ventromedial extent of the central nucleus of the inferior colliculus, bilaterally. In similar preparations that were unilaterally labyrinthectomized, this projection area is seen in the colliculus contralateral to the stimulated ear, but is substantially diminished in density, or altogether absent in the colliculus ipsilateral to the intact ear. A variety of tones spanning at least 3 octaves located in the center of the rat's auditory range suprathreshold, indicating that ipsilateral suppression of activity in the VM is broadly tuned. To further investigate the effects of unequal auditory activity on the labelling of the VM band, some animals were unilaterally labyrinthectomized with the intact ear left acoustically unstimulated. Again, dense labeling was seen in the VM contralateral to the stimulated ear, whereas no activity was seen in the VM ipsilateral to the intact ear. The above results demonstrate that the VM can be strongly inhibited ipsilaterally by unequal acoustic stimulation, or spontaneous activity arising from the 8th nerve. These cases by themselves do not show that activity within the auditory system is necessary for VM activity, since the VM could possibly receive a feedback input from extra-auditory sources. That the VM area requires auditory input for its substantial activity was demonstrated in 1. spontaneous activity in the 8th nerve was eliminated by bilateral cochlear destruction. In these cases the labelling in the VM area was bilaterally eliminated. 2. spontaneous activity originating in 8th nerve was necessary for VM activity. In summary, these experiments show the existence of a new and discrete collicular projection field having unique functional characteristics that are different from auditory projection areas so far reported.

Supported by grants RT1208, NS13052-02, and NS10414.
RESPONSES OF SINGLE COCHLEAR NUCLEUS NEURONS OF HORSESHOE BATS TO SINUSOIDALLY FREQUENCY AND AMPLITUDE MODULATED SIGNALS.  

Marianne Vater* (SPON: W.J. THOMPSON). Dept. of Biology, Frankfurt University, FRG.

The greater horseshoe bat, Rhinolophus ferrum-equinum, emits a low constant frequency component (CF) of about 85 kHz during echolocation. The echoes are hopper-shifted upward in frequency due to the relative movement between the bat and target. The bat compensates for these deviations in the echo frequency by inverting the frequency of its emitted calls so that the echo frequency remains constant within a narrow frequency band. The wing beats of prey insects result in periodic frequency and amplitude modulations of this carrier frequency which can be used by the bat as clues for detection and probably for the identification of moving prey objects. In order to examine the neuronal encoding characteristics of these complex time varying signals within the ascending auditory pathway of horseshoe bats, recordings were made from single cochlear nucleus (CN) neurons. The sample comprised neurons from all subdivisions of the CN-complex. Variable parameters of the sinusoidal frequency and amplitude modulated (SPM and SAM) stimuli were presented. The discharge patterns of most tonic units faithfully reproduced the course of the periodic modulations. It can therefore be expected that signals with more complex temporal patterns, as for example generated by predation of insects, will also be processed with only little distortion and are relayed as input information to higher centers. Phasic neurons did not preserve the stimulus time structure: they responded with a transient discharge activity to distinct portions of the modulation cycle. Synchronization was present over a wide range of signal intensities and modulation depths. Energization of frequency parameters for evaluations of the target distance and size. The sharply tuned CN "filter" neurons, tuned to 78-88 kHz, are able to encode modulation depths as small as ±50 Hz. The response properties to SPM signals. This becomes clear in the responses to different modulation rates. Primary-like neurons in the anterocentral CN are able to synchronize to rates as high as 500 Hz. Thus the stimulus coding in the time domain therefore exceeds the frequencies of prey insect wing beat frequencies (i.e., between 30 and 100 Hz). Build up neurons in the dorsal CN were unable to follow the periodic modulations in the frequency range tested (minimum modulation rate of 20 Hz). Phasic neurons in the dorsal CN typically lock only at modulation rates below 30 Hz, a behavior also reported for inferior collicular neurons, but a range largely covering the biologically important range of insect wing beat frequencies.

SPIRAL GANGLION NEURONS FOLLOWING DRUG-INDUCED ORGAN OF CORTI LOSS.  


The guinea pig organ of Corti was destroyed by a single treatment of ototoxic kanamycin following by ethacrynic acid (West et al., Arch. Otolaryngol., 98:32-37, 1973). The resulting loss of spikes at all frequencies tested suggests that the spiral ganglion neurons contributing to the cochlear nucleus are inactivated. Neurons in the dorsal CN typically respond to low frequency sounds, and their discharge patterns were studied in 10-µm serial sections of 23 ears from 15 animals, including 2 normals and 13 with post-treatment neurons. The resulting findings were the following: Neurons were unable to follow tones of frequencies below 300 Hz, a behavior also reported for inferior collicular neurons, but a range largely covering the biologically important range of insect wing beat frequencies.
Supported by PHS grants 1 F32 NS 05910-01 and 5 R01 NS14354.

Errors due to the finite length of the noise sequence and the convolution eliminates errors from the odd-order terms.

The lateral branch gives rise to thin, wavy terminals in the external ear canal of the animal seen about 10 minutes after infusion was stopped. Recovery began approximately 40 dB above threshold were reduced by up to 70% in a dose-dependent manner by propranolol. A half-maximal effect was immediately thereafter but was never complete within 2 hours. No immediate thereafter but was never complete within 2 hours. No consistent dose-dependent effects on CM were seen. Since comparable reductions of N1 and BSER responses were obtained at all dose rates, a major portion of the effects of propranolol in these experiments must be mediated within the cochlea. Although most of the effect on brain stem responses can be accounted for by a reduction in auditory nerve input to the CNS, the data do indicate some additional response reduction within the brain stem.
111 **EXTERNAL NUCLEUS OF THE INFERIOR COLLICULUS: A SITE OF OVERLAP FOR ASCENDING AUDITORY AND SOMATOSENSORY PROJECTIONS IN THE MOUSE.**


We have examined second-order auditory and somatosensory pathways which project to the external nucleus (EN) of the inferior colliculus (IC). The cells of origin that project to EN have been identified in the dorsal and ventral thalamic nuclei (DN and VN, respectively). In the contralateral dorsal column nuclei (DCN), retrograde transport of HRP labeled fusiform cells of the fusiform cell layer, and elongate, horizontal and portioned cells of the pretectal layer was demonstrated. In addition, HRP-labeled fusiform cells were concentrated around the margins of the contralateral dorsal column nuclei (DCN); large HRP-labeled fusiform cells were also observed within these nuclei. Finally, HRP-labeled fusiform cells were localized along the lateral border of the ansa intercolinella (AIC). In the contralateral trigeminal was determined.

In order to evaluate the extent of terminal-field overlap from these auditory and somatosensory projections, and to confirm that our HRP data are not due to HRP diffusion into adjacent IC subdivisions, we have examined the projections from DCN and VN(C) using the Pink-Heimer method for anterograde degeneration. (Analysis of the trigeminal projection to EN is still in progress.) Lesions of DCN or sections of the dorsal acoustic area produced dense fiber and terminal degeneration in the central nucleus (CN) and moderate degeneration in EN. Peak argyrophilic occurred 24 hr after the lesions, appearing uniform in density throughout the nucleus. Many degenerating fiber profiles were seen to bifurcate and then collateralize in both CN and EN; the overriding cortical was free of degeneration. Lesions of the trapezoid body produced a complex pattern of degeneration within EN. At short survival times (24-48 hrs), terminal degeneration was concentrated into patches, separated by degeneration-free zones. Appearance of the degeneration was associated with islands of cell-dense regions; the degeneration-free zones were associated with the intervening cell or space areas. At longer survival times (~ 500 um) filled the spaces between the terminal fields. Degeneration was heaviest rostrally, and gradually thinned out caudally. The degeneration CN and degeneration CN was determined.

In summary, EN of the mouse may be characterized not only by its cytoarchitecture, but by the pattern of afferent input from DCN and VN(C) as well. Although neither the density nor spatial distribution of these projections are identical, their terminal fields overlap within EN. Whether the heterotopic terminals actually synapses on the same EN neuron remains to be determined.

(Supported by NIH Grant NS 13126)

112 **COMPARISON OF INFERIOR COLLICULUS NEURONAL RESPONSE PROPERTIES IN C57BL/6 AND DBA/2 INBRED HOUSE STRAINS.**


Response properties of neurons in the inferior colliculus (IC) for inbred house strains—DBA/2 mice, which are innately susceptible to audiogenic seizures and noninnately susceptible C57BL/6 mice. The IC was chosen for study since recent studies to play a critical role in audiogenic seizures. Using tranquilized mice, extracellular single-unit recordings were made in the IC from IC neurons in DBA/2 mice. However, a small proportion of neurons in the control strain displayed dramatically abnormal response properties. These neurons showed sustained discharges with prolonged afterdischarges when stimulated with 200 ms tones of relatively high intensities. In some cases, increased levels of neural activity lasting for up to several seconds were recorded. These neurons were associated with exposure to tones of 60 db SPL. Most neurons with elevated neural activity were also found in the ventralateral region of the IC and the thalamus. After stimulation of such terminals generated a homogeneous distribution in EN. At short survival times (24-48 hrs), the tracer was injected with pressure. The IC was chosen for study since it appears to be sensitive to differences in the time of arrival of the frequencies delivered to the two ears, i.e. a stimulus which evokes the interaural delay paradigm. Over 90% of the cells that show agreement between the shapes of the curves obtained at low beat frequencies to the two ears, we could also study the natural response properties of the neurons in these two preparations. With the two stimuli, i.e. a stimulus which evokes the interaural delay paradigm to study the phase sensitivity of neurons in the inferior colliculus. (Supported by N.I.H. grants NS12732 and EY02606)

113 **LAMINAR CONNECTIONS IN THE CAT’S AUDITORY CORTEX (AI).**


Neurons in all areas of the cerebral cortex are organized in layers and this laminar pattern is related directly to the connections established by a given area. To examine the connections of layers in the cat’s primary auditory cortex (AI), we have recorded binaural responses (Cohen and Allard, 1979) and autoradiography for anterograde mapping of neuronal connections. Autoradiographs filled the spaces between the terminal fields. Degeneration was heaviest rostrally, and gradually thinned out caudally. The degeneration CN and degeneration CN was determined.

In summary, EN of the mouse may be characterized not only by its cytoarchitecture, but by the pattern of afferent input from DCN and VN(C) as well. Although neither the density nor spatial distribution of these projections are identical, their terminal fields overlap within EN. Whether the heterotopic terminals actually synapses on the same EN neuron remains to be determined.

(Supported by NIH Grant NS 13126)

114 **BINAURAL INTERACTION IN THE CAT INFERIOR COLLICULUS STUDIED WITH INTERAURAL DELAYS AND BINAURAL BEATS.**

T.C.T. Yin, S. Kuwada*, and R.E. Wickesberg. Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, Wis. 53706.

Host cells in the inferior colliculus receive afferent input and many of the low frequency cells (~3000 Hz) have been shown to be sensitive to differences in the time of arrival of the sound from the two ears. These differences specify the phase of the sound wave, i.e. the phase relationship obtained from a sound source moving in space. By changing the beat frequency or by interchanging the same information as the interaural delay paradigm. Thus, the binaural beat stimulus provides a means for gathering information about the frequency cells that receive binaural input to the interaural delay and binaural beat stimuli. Over 90% of the cells that show phase sensitivity will respond reliably to both stimuli. With the binaural beat stimulus the neurons in the latter strain display a group-specific delay which can be measured in degrees of frequency to the two ears, i.e. a stimulus which evokes the interaural delay paradigm. The IC was chosen for study since it appears to be sensitive to differences in the time of arrival of the frequencies delivered to the two ears, i.e. a stimulus which evokes the interaural delay paradigm. Over 90% of the cells that show agreement between the shapes of the curves obtained at low beat frequencies to the two ears, we could also study the natural response properties of the neurons in these two preparations. With the two stimuli, i.e. a stimulus which evokes the interaural delay paradigm to study the phase sensitivity of neurons in the inferior colliculus. (Supported by N.I.H. grants NS12732 and EY02606)

Anurans (frogs and toads) are particularly useful for studies of the specificity of neural connections in the auditory system. Their two auditory organs, the amphibian and basilar papillae, are sensitive to distinct frequency ranges and project onto separate populations of postsynaptic cells in the central auditory system. When the eighth nerve is severed, the axons regenerate and re-enter the brainstem. As part of a set of experiments concerning specificity of reinnervation, we have studied the response properties of regenerated auditory nerve fibers and their course through the central nervous system to their target sites.

The eighth nerve was severed unilaterally and the animals (Bufo americanus and Rana pipiens) were allowed to recover. At postoperative periods of 3 months or more, the responses to acoustic stimuli were recorded from the regenerated auditory nerve fibers by means of fluid-filled microelectrodes. Following the recording session the inner ear was opened, the posterior branch of the eighth nerve was cut, and horseradish peroxidase (HRP) was applied to the stump. The animals were perfused 2 days later and their brains processed for HRP labeling.

Fibers from both the amphibian and basilar papillae were encountered in the regenerated eighth nerve, indicating that neurons innervating both organs are capable of survival after axotomy and subsequent regeneration. Thresholds, latencies and tuning curves of the regenerated fibers appear to be normal. Histological analyses of the HRP-filled axons in the posterior branch (which contained fibers from the two auditory organs and the posterior semicircular canal) of control animals indicates that this branch normally runs only in the dorsal portion of the nerve as it approaches the medulla. In regenerated nerves, even though there is substantial twisting in the regrowth process, the fibers from the posterior branch remain in proximity to each other; these fibers are capable of regeneration to their original target neuropil even if they penetrate the brain via aberrant entry points. Once the fibers re-enter the vicinity of their postsynaptic cells, they display axonal varicosities (presumptive loci of synaptic terminals) typical of normal eighth nerve fibers. Preliminary recordings in the basilar papillae demonstrate that central auditory neurons can be driven by acoustic stimuli via the regenerated nerve, thus verifying that its auditory fibers remake functional connections.

Supported by NIH grant NS-09244


In the mustache bat the nuclei of the lateral lemniscus stand out as three large and cytoarchitecturally distinct areas, called the dorsal (DNLL), intermediate (INLL), and ventral (VNLL) nuclei of the lateral lemniscus. The cytoarchitectural appearance of DNLL and VNLL suggests that these nuclei have homologues in other mammals, but INLL appears so cytoarchitecturally unique as to question whether it is really part of the auditory pathway.

We studied the connections of these nuclei by placing electrophoretic deposits of horseradish peroxidase (HRP) or [3H]-leucine in these nuclei or in subdivisions of the cochlear nucleus, superior olivary complex or inferior colliculus. Evidence from anterograde transport shows that the anteroventral cochlear nucleus supplies the main ascending projection to both VNLL and INLL, but there are little or no projections from the cochlear nucleus to DNLL. Deposit of HRP within the borders of VNLL reveal large numbers of labeled cells in the anteroventral cochlear nucleus; in the posteroventral cochlear nucleus only octopus cells were labeled. Deposits of HRP within INLL also revealed labeled cells throughout the anteroventral cochlear nucleus; in the posteroventral cochlear nucleus only octopus cells were not labeled, but other types of cells were. Very few labeled cells were found in the dorsal cochlear nucleus after deposit of HRP in any part of the lateral lemniscus.

Anterograde transport of [3H]-leucine shows that the superior olivary complex projects ipsilaterally to all three nuclei in the lateral lemniscus and contralaterally to DNLL. The HRP evidence shows that the cells of origin of projections to VNLL and INLL are located in perfolitary cell groups and in the medial nucleus of the trapezoid body. The lateral nucleus of the trapezoid body projects almost exclusively to INLL. The medial and lateral superior olives appear to project mainly to DNLL. Finally, all three nuclei project to the ipsilateral inferior colliculus, mainly to the central nucleus, and VNLL sends a major projection to DNLL and inferior colliculus of the contralateral side; INLL and VNLL project to the medial geniculate body.

We conclude first that all three nuclei of the lateral lemniscus in the mustache bat are major components in auditory pathways ascending to the inferior colliculus and thalamus and, second, that each of these nuclei can be distinguished from one another on the basis of their connections.

[This research was supported by the National Science Foundation.]
AUTONOMIC FUNCTION

A study was made to determine the origin of the 2-6 c/s rhythm in preganglionic splanchnic and postganglionic renal sympathetic nerve discharge (SND) in the chloralose anesthetized, baroreceptor denervated cat. Specifically, it was of interest to learn whether the rhythm is inherent to the brain stem network responsible for the SND or whether it is introduced by the vagus nerve. Data generated by Egan and Cramer et al. (Pflügers Arch. 370: 221-225, 1977), it is dependent upon the integrity of interconnections between the forebrain and brain stem. For these purposes, the frequency characteristics of SND and cortical activity (parietal-frontal EEG) were compared using crosscorrelation and power spectral analyses before and after baroreceptor denervation and after midcorticomedullary decerebration. Crosscorrelation analysis failed to reveal a relationship between SND and EEG. SND laggged cortical activity by an average of 30 ms. Power spectral analysis revealed a postulated pre-synaptic origin of the 2-6 c/s rhythm in the brain stem. (Supported by PHS Grant HL-13187.)

EVIDENCE FOR PRE-SYNAPTIC α-ADRENERGIC INHIBITION OF A SYMPATHETIC-CHOLINERGIC SYSTEM. Patricia J. Bernthal*, Joanne L. Moore and Michael C. Koss. Dept. Pharmacology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

As early as 1929, Lilley proposed that epinephrine has a modulatory effect on the sympathetic-cholinergic efferent system in humans (Billigheimer, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.. 1929, 125: 104). Previous studies in our laboratory indicated that systemic administration of epinephrine depresses the amplitude of postganglionic evoked electrodermal responses in cats. This effect was observed by 10 mg/kg and was independent of the side of injection. The complex rhythm in SND of the baroreceptor denervated and decerebrate cat was transformed into a simple rhythm (i.e., constant interburst intervals) which eliminated the upper bound of the 2-6 c/s band during short epochs (15-30 s of aephythm). Furthermore, the rhythm was synchronized to single shocks applied to the cardiovascular reactive sites in the medulla and to afferents from the sciatic nerve. The results indicate that normally entrained to each other, the 2-6 c/s rhythms in SND and EEG are independently generated. That is, the 2-6 c/s rhythm in SND is not dependent upon the integrity of interconnections between the forebrain and brain stem. Rather, the results, in conjunction with an earlier report (McCall and Gepper, Brain Research 139: 139-143, 1975) which eliminated the spinal cord as the site of origin of the 2-6 c/s rhythm in SND, indicate that the rhythm is generated in the brain stem. (Supported by FHS Grant NS-13187.)

SYMPATHETIC FUNCTION IN A SYMPATHETIC CANALIZATION AND ITS POTENTIAL SIGNIFICANCE IN ESSENTIAL HYPERTENSION. R. I. Birks, Physiology Department, McGill University, Montreal, Canada H3G 1Y6.

Fainting of the preganglionic neural input to the cat superior cervical ganglion into brief high frequency trains causes a progressive and maintained increase in ganglionic acetylcholine acetylcholine (ACh) turnover up to 1700 fold above control during sympathetic ganglionic nerve stimulation in chloralose anesthetized cats. Elapsed sodium loading when the sympathetic ganglionic nerve was inhibited, but only during accelerated sodium pumping in the recovery period, it is concluded that the effect is related to accelerated sodium pumping, rather than to a direct effect of the raised intracellular Na⁺ at the nerve terminals. ACh output was found to increase 2.5-3.0 fold during 60 min following by patterned stimulation, or by equally spaced low frequency pulses during recovery in normal K⁺ following pump inhibition. These effects on ACh output are greater than can be accounted for by the increase in ACh stores (Birks, R.I. J. Physiol. 271, 847-862, 1977). Because ACh release is known to be increased by raised [Na⁺] (Birks, R.I. and Cohen, M.W. Proc. Roy. Soc. B. 170, 401-414, 1967; Leeson and Attwood, J. Physiol. 138, 367-371, 1957) it is proposed that the extra effect of patterned stimulation on ACh output is related to the increase in [Na⁺] and perhaps also to the hyperpolarization which accompanies accelerated sodium pumping. The general conclusion that arises from this work is that the internal sodium load at synapsing preganglionic nerve endings modulates ACh output and thus effecter response.

Recent work indicates that an abnormally high passive permeability of Na⁺ to Na⁺ is an important genetically determined defect in essential hypertension (see Garay, R.P. and Meyer, P., Lancet, Feb. 17, p. 349-353, 1979 for ref.). This membrane defect imposes an increased intracellular sodium load on affected cells, and if present at sympathetic neurons, would be expected to promote excessive sympathetic activity and thus increase vascular smooth muscle tone thereby contributing to hypertension (Birks, R.I. and Levine, S.S. in preparation).

Supported by the Muscular Dystrophy Association of Canada.

A laser-Doppler, blood flow monitor and an LED-phototransistor reflection radiation system have been specifically used to monitor the dynamics of the microvascular network in skin of human finger and forearm and exposed rat muscle. These noninvasive techniques measure changes in blood flow using the Doppler shift of red blood cell velocity within small (mm²), superficial regions of the microvasculature. The Doppler-broadening of laser light scattered by moving red blood cells is analyzed by a microprocessor which is felt to be linearly correlated with flow (J. Physiol. 232:441-8, 1977). The radioenzytic technique employs a calibrated near-infrared light source and phototransistor to detect light backscattered from underlying tissue (Techniques in Psychophysiology, 1979). The radioenzytic signal (volatile reduced to tissue blood flow) in six male subjects (aged 50-75 years) was measured by the radioenzymatic method.

1) Maximum laser-Doppler blood flow values were 2.7 ± 0.5 g/min of pulseless component or 11 ± 3% from the fingertip and 2.5 ± 0.1 g/min from the forearm skin. The corresponding radioenzytic values were 15 ± 5 g/min with a fractional pulsatile component of 0.4 ± 0.7 from the fingertip and 5 ± 1 g/min with 0.04 ± 0.03% pulsations from the forearm skin. 2) During the Valsalva maneuver, the laser-Doppler blood flow values from the fingertip was transiently reduced to less than 10% of the resting value with an associated 5-7% increase in radioenzytic signal suggesting a decrease in local blood volume. 3) During graded, external-applied pressure increases, laser flow values increased in a sigmoidal manner with a corresponding increase in pulsatile flow. The radioenzytic signal corresponded to increase in average blood volume with an associated change in pulsatile flow. 4) During laser irradiation, a 6-fold increase in laser flow values was observed in the forearm skin. The magnitude of this observed hyperemic response was not significantly different from 15 to 20 min. In the rat muscle, autoregulation of blood flow was observed with partial aortic compression and hyperemic responses were measured following focal ischaemia.

These results demonstrate the applicability of the laser-Doppler and radioenzymatic techniques for continuous, noninvasive measurement of microvascular dynamics in normal and diseased tissue. By providing a rapid, continuous, indirect index of these dynamics, the techniques should prove useful in the assessment of disorders of the adrenergic nervous system, and of intrinsic vascular and local regulatory factors.

REDUCED PRESSOR RESPONSE TO INTRACAROTID ANGIOTENSIN INFUSION AFTER SUBFORNICAL ORGAN LESION IN RATS. J. Buggy, W.E. Wells, W.J. Bryan* and G.D. Fink*. Department of Physiology, University of South Carolina, Columbia, South Carolina 29208, and Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan, 48824

Blood-borne angiotensin (AII) may rapidly increase arterial pressure not only by its direct vasoconstrictor action on vascular smooth muscle but also by activation of central nervous system mediated pressor mechanisms. Although some evidence also implicates more rostral brain sites (area postrema and subnucleus medialis) in the mediation of central AII action, it is now generally accepted that there are a significant number of postganglionic sensory fibers in the cerebral sympathetic trunk. This work is partially supported by Public Health Service grants HL 07859 and HL 09611, a grant from the University of Texas Heart Association, and research assistantship of the University of Texas Medical Branch.

AFTER SUBFORNICAL ORGAN LESION IN RATS. J. Buggy, W.E. Wells, W.J. Bryan* and G.D. Fink*. Department of Physiology, University of South Carolina, Columbia, South Carolina 29208, and Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan, 48824

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Clonidine, a potent antihypertensive agent, has been demonstrated in our laboratory to exert wide-ranging and potent cardiovascular actions via the activation of α-adrenoceptors in the medullary reticular formation (MRF) (Chen and Chan, Neuroscience Abst. 2:18, 1978) in normotensive cats, activating cardiovascular responses in the presence of vagus nerve. In addition, the MRF has been demonstrated in the rat to be at least one of the central sites of clonidine induced actions (Chan and Koo, Neuropharmacol. 17:367, 1978).

The present study was designed to investigate cardio-vascular effects of clonidine in experimentally-induced hypertensive cats and the involvement of MRF in these actions.

Cats that were fasted for 24 hours and maintained on 10% dextrose and saline (55 µg/kg, i.p.) or precociously decerebrated were used. Cannulations of left carotid artery and left femoral vein were performed for measurement of arterial blood pressure (ABP) and injection of drug. Heart rate (HR) was determined by conventional EKG. Experimental hypertension was induced at the beginning of the recording session, which was followed by a maintained elevation of ABP without a significant change in HR.

Systemic injection of clonidine (10 µg/kg, i.v.) in the experimentally-induced hypertensive cat produced an initial, transient increase in ABP (mean, systolic, and diastolic), followed by a significant decrease of the same parameters and a prolonged suppression of HR (old = ∆29±9 vs adult = ∆88±6 bpm) and a transient hypertension, which represents a peripheral vascular effect of clonidine, depression of ABP and HR by this imidazoline compound was eliminated after bilateral carotid cannulations where these cardiovascular events were mediated by the vagus nerve. Likewise, bilateral focal electrolytic lesions of MRF (three 1 mm apart lesions on each side) resulted in suppression of hypertension and bradycardia after systemic injection of clonidine (10 µg/kg). Preliminary results indicated that unilateral microinjections of clonidine (10 µg/Kg) into three 1 mm apart loci in MRF at an ineffective dose (0.2 µg/Kg) when administered systemically produced a significant decrease of ABP without the presence of the hypertensive and control microinjections of saline (vehicle) did not produce any cardiovascular changes.

It is concluded that clonidine may exert its central actions in experimentally-induced hypertensive cats via activation of medial medullary reticular neurons to produce depression of ABP and HR, an action that would require the presence of vagus nerve.


The canine area postrema (AP) has been shown by Barnes et al. (Circ. Res., in press) to be the site of a physiological pressor pathway. We have previously described the morphological compos­ition of this structure (Chernicky et al. Am. J. Anat. 110:1-18, 1976). In order to demonstrate the anatomic pathway from the canine AP, efferent projections were studied by the Fink-Heimer and Witanowski-Doox silver staining degeneration technique. Discrete electrocoagulation lesions were placed in the AP under direct vision with the aid of an operating microscope. Following 5-7 days, animals were perfused with isotonic saline followed by 10% buffered formalin. After 4-6 weeks storage in 10% formalin, the brains were serially sectioned at 25 µm in the sagittal, horizontal, or transverse plane. Every fifth section was stained with luxol fast blue and/or cresyl violet for both anatomic orientation and establishment of the extent of the lesion. Adjacent sections were silver stained for degenerating axons and terminals.

The most prominent projection from the canine AP was through the ipsilateral mesencephalic trigeminal nucleus (MTN), previously described in the cat by Aarest (Am. J. Anat. 107: 291-303, 1960). The amount of degeneration seen in this bundle was constant throughout its length from the obex to the most rostral portions of the AP. A sparse projection was seen to terminate in the ventromedial portion of the ipsilateral nucleus tractus solitarii (NTS). A small bundle of degenerating fibers crossed the nucleus commissuralis of Cajal, entering the region of the contralateral NTS just caudal to the obex. A few scattered terminals were observed in the contralateral AP-NTS junctional zone and in the dorsolateral corner of the AP.

The observation of terminal degeneration in the AP-NTS junctional zone confirms the finding in Golgi studies of AP neurons with short axons projecting directly into the NTS. The present study also demonstrates an efferent projection from the AP to the medi­al NTS. The degenerating fibers seen crossing the midline in the NTS pointed caudally to the raphe nuclei and rostrally to a bundle within the APNTS interconnecting of the two halves of the AP. This study sug­gests that the AP projects only to the closely adjacent medul­lary structures, and implies that the AP-NTS facilitatory effec­tive effects on the sympathetic vasomotor outflow are multi­synaptic.

Supported by grants from NIH, HL-64335; American Heart, 764 46; and the Reinberger Foundation.


The blood pressure and heart rate of freely moving or restrained male Fisher-344 rats at 3 months (young), 12 months (adult) and 28 months (old) were monitored through a chronically indwelling catheter which was implanted in the ventral tail artery at least 20 hrs before the experiment (Chieh and Kopin, Amer. J. Physiol. 234:H653, 1978). The simultaneous stress activity in the animal were determined by radioenzymatically measuring the catecholamines in 50 µl of plasma. Concomitant and unstrained Fisher-344 rats were handled similarly and stressed in heart rate, systolic and diastolic blood pressure with increasing age. The heart rate of old, 369±12 b/min, was significantly lower than those of young (396±6 b/min) or adult (385±5 b/min) rats. Systolic and diastolic blood pressure of unstrained young, adult and old rats were 124±5/71±4, 141±3/78±2 and 120±4/92±4 mm Hg, respectively. A 3 min or 20 min forced immobilization was applied to the animals and used as physical and emotional stressors in order to investigate the effect of the aging process on the amplitude and rate of responses in the sympathetic and cardiovascular systems. Three minutes of stress failed to increase the blood pressure and heart rate of old rats, but increased cardiovascular responses in young and adult rats. Following 30 min of stress, the heart rate of aged rats increased slightly (29±9 b/min), approximately 1/3 to 1/2 of the increase seen in young rats. The immobilized rats had similarly lower baseline THS levels than unstrained controls because of their massive release of epinephrine following the 30 minute stress. The resting plasma levels of norepinephrine in unstrained Fisher-344 rats ranged from 0.6 to 2.6 ng/ml and increased slightly with increasing age. Aged rats showed no decrease in the maximal adrenergic release due to stress, but had a prominent delayed increase in the catecholamine release. The respective increments of plasma norepinephrine after 3 min and 30 min stresses were 1.8±0.5 and 3.5±0.4 ng/ml. The delay of norepinephrine release may be especially β receptors, in the target organs was evident because the 30 minute stress induced plasma epinephrine in old rats (17.3±1.1 ng/ml) than in adult rats (34.3±0.6 ng/ml) while producing a decrease in β-adrenergic responses; i.e., heart rate old = 239±9 b/min and blood sugar old = 141±3 vs adult = 217±5 mg/ml. The present results indicate that the release mechanism of the sympathoadrenal medullary system of aged Fisher-344 rats may not respond maximally but not efficiently to stress due to a decline in the rates of catecholamine release and a decrease in the activities of adrenergic receptors or effectors in the target organs.


Although it is known that the function of the hypothalamus can be affected by cardiovascular afferent information in the buffer nerves the course and distribution of these afferent fibers from the first relay in the n. tractus solitarii (NTS) to the hypo­thalamus is not known. The traditional view is that the NTS receives afferent input from several sources, including the vagus nerve and the baroreceptors. The present study demonstrates that these fibers terminate in a discrete, physiologically competent nucleus located in the ventromedial portion of the hypothalamic region, termed the ventromedial hypothalamic nucleus (VMH).

The hypothalamus was studied in anesthetized cats. A small bundle of degenerating fibers crossing the midline of the NTS projecting to bilateral VMH was found in each cat. The present study demonstrated the existence of a functional, probably bilateral NTS-VMH pathway of afferent information directly to the hypothalamus.

(Supported by MRC of Canada)
A study was undertaken to compare regional cerebral blood flow in normal subjects and migraineurs experiencing the effects of biofeedback induced skin temperature changes. Also we investigated the specific and nonspecific effects of biofeedback training on the frequency, intensity and duration of migraine headaches.

A typical classic migraine attack is characterized by a biphasic pattern of autonomic behavior involving vasodilatation of the intracranial and extracranial arteries. It has been hypothesized that hand-warming through bio-feedback training may result in a decrease in sympathetic outflow, thereby interrupting the vasomotor pattern of change in a migraine headache.

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

Each subject was subsequently given 2 measures of regional cerebral blood flow to determine the autoregulatory ability. One was utilized in a steady-state condition and a second while subjects were attempting to manipulate their skin temperature in the directed direction.

Results show small, statistically non significant, changes in blood flow for normal volunteers whether they cool or warm their hands. In contrast, migraineurs increase the non-dominant left hemisphere flow 8% with suggestive increases regionally distributed. Examination of the data shows typical and non specific effects on frequency, severity and duration of headache. This study confirms autonomic peculiarities in migraineurs which result in novel changes in blood flow and warming or cooling of the dominant hand. It is proposed these changes explain the reported salutary effects of hand-warming in migraine.

The presence of non specific effects of biofeedback training emphasizes that placebo effects play an important part in relieving symptoms of migraine.

CELLS OF ORIGIN OF MOTOR AXONS AND AFFERENT DISTRIBUTION OF THE SUBDIAPHRAGMATIC VAGUS IN THE RAT. Janet D. Coil* and Ralph N. Nauta. The Rockefeller University, New York, NY 10021.

The neurons of origin of preganglionic motor axons that travel in the subdiaphragmatic segment of the vagus nerve classically have been ascribed to the dorsal motor nucleus of the vagus (DMX) in the medulla. To re-examine the central cell groups that give rise to such axons in rats, the ventral or dorsal branch of the subdiaphragmatic vagus was transected near the stomach and incubated in crystalline horseradish peroxidase (HRP) for 6 to 8 hours. After 48 hours post-operative survival, the animals were killed and their brains processed according to standard HRP histochemical technique, using tetramethyl benzidine as the chromagen. After counterstaining with neutral red, the tissue sections were examined under bright- and dark-field illumination for the presence of HRP-positive somata. As expected, numerous retrogradely labeled cells were present in DMX, distributed throughout the rostro-caudal extent of the nucleus. HRP-positive cells were also observed bilaterally in the rostral portion of the nucleus ambiguous.

The presence of autonomic imbalances may be important in the development of autonomic dysfunction. This is in contrast to previous accounts in which motor fibers of the nucleus ambiguous were considered to project only to the larynx, pharynx and cervical esophagus (Lawn, J. Comp. Neurol. 127, 1966, p. 293), but support functional evidence that suggests DMX is not the exclusive source of subdiaphragmatic vagal motor fibers (Kerr, J. Physiol., 202, 1969, p. 755; Lawn, J. Physiol., 174, 1964, p. 232.) Different patterns of labeling could be traced to a circumscribed portion of the nucleus tractus solitarius immediately ventral and rostral to the area postrema.


Stress-induced cardiac arrest and myocardial pathology observed in squirrel monkeys has been suggested to be mediated by the autonomic nervous system (Psychophysiol. 14: 322, 1977). The involvement of parasympathetic cardiac input was studied by bilateral cervical vagotomy. This procedure in 27 monkeys was accomplished in one operation. While 9 monkeys succumbed suddenly within 24-48 hrs, no additional deaths over 35±13 days (Ms±SE) occurred. Lethargy and anapnea were almost totally eliminated by vagotomy in two operations with weight stabilization used to indicate readiness for second operation. Since the only mortalities were two monkeys that had a left followed by right section, the sequence of vagi section was important. Because the right vagal input is greater than the left, the effect of a right-left denervation was more evenly distributed between the two operations than a left-right denervation. This observation suggested autonomic imbalance may be a factor in vagotomy deaths. Seventeen monkeys after the two stage vagotomy were studied for 40±8 days before stress with no other mortalities. While resting heart rate (335±4 bpm; N=30) was greater than that of intact monkeys (300±8 bpm; N=28; p<.01), blood pressure did not differ (vagotomy: 151/120mm Hg; N=19; intact: 152/120mm Hg; N=19). Tachycardia followed by bradycardia with cardiac arrest which had an incidence comparable to that of intact monkeys is suggested by the abrupt removal of vagal restraint. Since no fibrosis occurred in intact monkeys after a 24-hr. stress, cardiacmyopathy was attributed to vagotomy per se and enhanced sympathetic activity. Since no fibrosis occurred in intact monkeys after a 24-hr. stress, cardiacmyopathy was attributed to vagotomy per se and enhanced sympathetic activity. Since no fibrosis occurred in intact monkeys after a 24-hr. stress, cardiacmyopathy was attributed to vagotomy per se and enhanced sympathetic activity. 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THE EFFECT OF LEFT CARDIAC SYMPATHETIC VAGOTOMY ON CARDIAC CHANGES IN NORMAL SUBJECTS DURING BEHAVIORAL STRESS IN DOGS. Lewis K. Clarke* and Richard A. Calloway, Dept. of Physiol, Univ. of Texas Haa. Sci. Ctr. at Dallas, TX 75235.

Alterations in heart rate (HR), left ventricular systolic pressure (LVSP), and maximum left ventricular pressure development (LV dp/dt max) during a 13 day Sidman shock avoidance task were studied in 3 groups of four chronically prepared dogs. In one group of animals the left dorsal and ventral cardiac subclavian nerves were transected between the sternale and the caudal cervical ganglia resulting in a denervation of the left cardiac sympathetic nerves. The second group of dogs was a neurologically intact, experimental stress group, and the third group was a neurologically intact, nonstress control. The intact stress group demonstrated phasic increases in HR, LVSP, and LV dp/dt max during the avoidance period of each day as well as tonic increases in HR, LVSP, and LV dp/dt max during the 13 days of the experiment. In the denervated animals there was no evidence of phasic increases in any of the parameters during the avoidance period. Tonic levels of LVSP and LV dp/dt max in the denervated group were not significantly different from controls, but tonic levels of HR remained elevated. These results suggest that the integrity of the left cardiac sympathetic nerves is necessary for stress induced change in LVSP, LV dp/dt max, and phasic increase in HR during the avoidence period of each day. However, right cardiac sympathetic and/or vagal influences are apparently responsible for stress induced tonic changes in HR.

The right external jugular vein of five male Wistar rats was catheterized to permit intravenous injection of small volumes of blood from freely-moving rats. The flexible silastic catheter was encased in a protective steel wire sleeve which was anchored by a pulley to allow for relatively free movement. Plasma norepinephrine (NE) and epinephrine (E) concentrations were determined by the radioenzymatic assay system from Amersham. The animals were restrained 3 times and each experiment was separated by one day of rest. Restraint was done by securely taping the animal to the workbench for 30 min. Blood samples were drawn at 0 min in the home cage, at 1, 5, 15 and 30 min during restraint and at 60 minutes (30 minutes after the animal was released and returned to its home cage).

Animals differed behaviorally: excitement ranged from mild to severe during the first stress period and this behavior declined noticeably during the next two restraint periods. Levels of NE and E were similar in all animals at the beginning of the experiments, 186.6±40 and 101±29 pg/ml, respectively; baseline values at 0 time did not change for the next two experiments. Restraint stress markedly increased NE and E levels during the first exposure with peak levels of NE (1264±33 pg/ml; 576% as compared to 0 min) and E (1230±500 pg/ml; 111%) occurring at 5 min during the first restraint period. Plasma levels of NE (35±558 pg/ml) and E (288±137 pg/ml) were still significantly elevated 30 min after the stressor was removed, and E levels were greater than those seen with NE (about 500%). The second restraint period showed a decrease in peak NE (250%; p<0.1) and E (300%; p<0.05) levels. The third experiment demonstrated further adaptation since peak levels of NE (260%; p<0.05) and E (235%; p<0.05) continued to decline slightly. Studies of the effects of various drugs on the stress response are planned.

135 HYPERTENSIVE EFFECTS OF CEREBELLAR LESIONS IN THE FASTIGIAL NUCLEUS.  Kenneth J. Bommer, Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Colony-bred beagles were selected for long-term studies of arterial blood pressure (AP) both prior and subsequent to bilateral lesions in the rostral fastigial nucleus (FN) as an effort to examine the autonomic role of the nucleus in the conscious animal. Previous studies with dogs in the laboratory have described the hypertensive response to electrical stimulation of the FN in anesthetized and conscious dogs. The objective of the study was to verify the absence of mean AP changes following ablation of rostral FN. Four beagles (2 male, 2 female) were implanted under sterile procedure with solid state pressure transducers in descending aortae and AP and heart rate (HR) recorded on a monthly basis, for 24 hr sessions, up to 9 months following surgery. Animals were acclimated (1 month) to the laboratory environment and recording apparatus prior to chest surgery. The 24 hr studies were performed 2-3 months prior to lesioning and 1-4 months post-lesion. The 24 hr mean ± S.D. of all the mean AP's for 4 dogs was 102.6±4.7 mmHg and HR was 69±10.5 beats/min (ten-24 hr). All studies were performed 2-3 months prior to lesioning and 1-4 months post-lesion. The 24 hr mean ± S.D. of all the mean AP's for 4 dogs was 102.6±4.7 mmHg and HR was 69±10.5 beats/min (ten-24 hr). After the 24 hr study was performed, the animal was anesthetized with alpha-chloralose (110 mg/kg i.v.) and the regions of the rostral FN were stereotaxically implanted under sterile surgery with concentric bipolar electrodes. When the maximal pressor response was obtained, the site was then lesioned with RF current until the response could no longer be elicited. The 24 hr mean ± S.D. for 3 dogs beginning 1 month following surgery was 122±5.1 mm Hg and heart rate was 81±11.5 beats/min. All 4 dogs showed elevated mean AP and the fourth dog post-lesioning exhibited waking mean AP of 110 to 160 mm Hg, a pulse pressure of 175/110 and 100 mm Hg mean AP during sleep but the 24 hr mean was not yet available. These results suggest that a supramedullary level in some areas, there is projections of sinus nerve, sympathetic and parasympathetic nerve at the same place.

Supported by C.N.R.S. (AI)


Somatostatin (SS) and SS analogs, e.g. des-4d[Tyr^*][Pro^*]SS (ODT-SS) placed intracisternally (ic) or intracerebroventricularly (icv) have been demonstrated to prevent the hyperglycemia, hyperglucagonemia, and hypoinsulinemia induced by blood pressure. These agents may act within the brain to decrease sympathetic nervous system (SNS) activity. Therefore, we have carried out studies to determine whether SS and SS analogs prevent activation of the SNS by other neural stimuli. Plasma epinephrine (E) and norepinephrine (NE) were measured using a radioenzymatic assay, i.e. enzymatic conversion of [3H]-methyl-hydroxylated drug to radioactivity. Treatment with SS and SS analogs may act within the brain to decrease sympathetic nervous system (SNS) activity. Therefore, we have carried out studies to determine whether SS and SS analogs prevent activation of the SNS by other neural stimuli. Plasma epinephrine (E) and norepinephrine (NE) were measured using a radioenzymatic assay, i.e. enzymatic conversion of [3H]-methyl-hydroxylated drug to radioactivity. The Salk Institute, La Jolla, CA 92037.


The right external jugular vein of five male Wistar rats was catheterized to permit intravenous injection of small volumes of blood from freely-moving rats. The flexible silastic catheter was encased in a protective steel wire sleeve which was anchored by a pulley to allow for relatively free movement. Plasma norepinephrine (NE) and epinephrine (E) concentrations were determined by the radioenzymatic assay system from Amersham. The animals were restrained 3 times and each experiment was separated by one day of rest. Restraint was done by securely taping the animal to the workbench for 30 min. Blood samples were drawn at 0 min in the home cage, at 1, 5, 15 and 30 min during restraint and at 60 minutes (30 minutes after the animal was released and returned to its home cage).

Animals differed behaviorally: excitement ranged from mild to severe during the first stress period and this behavior declined noticeably during the next two restraint periods. Levels of NE and E were similar in all animals at the beginning of the experiments, 186.6±40 and 101±29 pg/ml, respectively; baseline values at 0 time did not change for the next two experiments. Restraint stress markedly increased NE and E levels during the first exposure with peak levels of NE (1264±33 pg/ml; 576% as compared to 0 min) and E (1230±500 pg/ml; 111%) occurring at 5 min during the first restraint period. Plasma levels of NE (35±558 pg/ml) and E (288±137 pg/ml) were still significantly elevated 30 min after the stressor was removed, and E levels were greater than those seen with NE (about 500%). The second restraint period showed a decrease in peak NE (250%; p<0.1) and E (300%; p<0.05) levels. The third experiment demonstrated further adaptation since peak levels of NE (260%; p<0.05) and E (235%; p<0.05) continued to decline slightly. Studies of the effects of various drugs on the stress response are planned.

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137 EFFECTS OF CENTRAL ADMINISTRATION OF ANGIOTENSIN II ON NOREPINEPHRINE INDUCED REFLEX BRADYCARDIA AND BARORECEPTOR PRESSOR RESPONSES IN CATS. J.S. Francis, A.S. Tadepalli and J.P. Buckley.* Inst. for Cardiovascular Studies, Univ. of Houston, Houston, Texas 77004.

Experiments were performed on cats anesthetized with chloralose-urethane, and artificially ventilated. Reflux bradycardia was induced by intravenous (I.V.) pressor doses of norepinephrine. Intravenous (IVT) infusion of angiotensin II (AI) or its antagonist saralasin (SAR) was performed through a cannula in the lateral ventricle and the fluid exited through the cisterna magna. Infusion of AI (2.4 µg) produced a 20-40% increase in arterial pressure (AP) and slight increase in heart rate (HR). During the infusion of AI and for 30-40 min after, the reflux bradycardia induced by I.V. norepinephrine was significantly enhanced by 2-4 fold but the pressor responses to I.V. norepinephrine were not altered. Also during this time the HR and AP increases normally seen during bilateral carotid occlusions (BCO) were enhanced by 53% and 46% respectively. Intrusion of the AI antagonist SAR (30-60 µg) IVT produced a significant lowering of AP but no change in HR. Pre-infusion of SAR blocked the AI induced increase in AP, and the enhancement of both the norepinephrine induced reflux bradycardia and the BCO pressor response. Systemic administration of AI on vagus in which induced an equal rise in AP as that seen with IVT administrated AI, produced little change in the reflex bradycardia and no change in the BCO response. These results suggest that AI enhances the reflex bradycardia and BCO response by an action in the central nervous system and that SAR can prevent these effects.


The cardiac-related rhythm in SND is transformed into a complex rhythm of brain stem origin (i.e., frequency of sympathetic nerve slow wave activity varies between 2-6 c/s during baroreceptor denervation. The present study was designed to determine the basis for the complex rhythm. For this purpose, discharges recorded simultaneously from different postganglionic sympathetic nerves were compared before and after baroreceptor denervation (i.e., bilateral section of carotid sinus, aortic depressor and vagus nerves). The phase relations between slow waves in postganglionic nerves (e.g., external carotid and renal) which exit from the same sympathetic ganglion (i.e., intraganglionic recording) were fixed before and after baroreceptor denervation. As a consequence, crosscorrelation functions approached a value of one. Crosscorrelation analysis revealed a weaker relationship between slow wave activity recorded from postganglionic nerves (e.g., external carotid and renal) which exit from different sympathetic ganglia (i.e., interganglionic recording). The weaker relationship in the experiments with interganglionic recording was explained by varied shifts in the phase relations between slow wave activity from cycle to cycle of SND. Regarding this point, the distribution of intervals between the peaks of slow waves recorded from the external carotid and renal postganglionic nerves in 100 consecutive cycles of SND was 8017 ms before baroreceptor denervation and 132±6 ms after baroreceptor denervation (6 experiments). Importantly, the shifts in phase relations between slow wave activity in the experiments with interganglionic recording far exceeded the range of conduction times in pathways from the brain stem to the external carotid and renal nerves (as determined from the range of onset latencies of sympathetic nerve potentials evoked by electrical stimulation of modulatory pressor sites). These observations have led us to propose that the brain stem sympathetic network is comprised of a number of coupled oscillators, each of which is inherently capable of producing a 2-6 c/s rhythm in sympathetic preganglionic neurons. Variations in the phase relations between slow wave activity in the interganglionic recording experiments further suggest that the complex form of the 2-6 c/s rhythm in SND results as the consequence of changes in the sequence of activation of coupled brain stem oscillators. That is, the leading brain stem focus is believed to shift from cycle to cycle of SND. (Supported by PHS Grant HL-13187.)

139 CENTRAL PHYSIOLOGICAL ORGANIZATION OF THE CARDIAC VAGUS. G. Steven Gels and Robert D. Warster. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153.

Previous studies in this laboratory localized cardiac vagal preganglionic somata in the dorsal motor nucleus of the vagus (DMN) and the nucleus ambiguous (NA) in the cat (Neuroscience Abstracts 4: 20, 1978). The present study was designed to investigate the role of each nucleus in cardiac control.

Occipital craniotomies were performed in anesthetized cats and stimulating electrodes were inserted into the DMN or NA on either side of the neuraxis. A strain gauge arch was sutured to the right ventricle and catheters were inserted into the right ventricle and the fluid exited through the cisterna magna. Cerebroventricular (CV) potentials evoked by electrical stimulation of medullary pressor sites). These observations have led us to propose that the brain stem sympathetic network is comprised of a number of coupled oscillators, each of which is inherently capable of producing a 2-6 c/s rhythm in sympathetic preganglionic neurons. Variations in the phase relations between slow wave activity in the interganglionic recording experiments further suggest that the complex form of the 2-6 c/s rhythm in SND results as the consequence of changes in the sequence of activation of coupled brain stem oscillators. That is, the leading brain stem focus is believed to shift from cycle to cycle of SND. (Supported by PHS Grant HL-13187.)

PHYSIOLOGICAL ORGANIZATION OF THE CARDIAC VAGUS. G. Steven Gels and Robert D. Warster. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153.

Previous studies in this laboratory localized cardiac vagal preganglionic somata in the dorsal motor nucleus of the vagus (DMN) and the nucleus ambiguous (NA) in the cat (Neuroscience Abstracts 4: 20, 1978). The present study was designed to investigate the role of each nucleus in cardiac control.

Occipital craniotomies were performed in anesthetized cats and stimulating electrodes were inserted into the DMN or NA on either side of the neuraxis. A strain gauge arch was sutured to the right ventricle and catheters were inserted into the left and right ventricular chambers for monitoring left and right ventricular pressures, respectively. The strain gauge output and the rate of rise of the left ventricular pressure curves (dP/dt) served as indices of ventricular contractility (VC). Electrode tip locations were histologically verified.

DMN stimulation produced decreases in mean arterial blood pressure (MAPB), dP/dt and strain gauge output; no change in heart rate (HR) and increases in left and right ventricular end-diastolic pressures (LVDEP, RVDEP). NA stimulation produced decreases in HR and MAPB and increases in dP/dt, strain gauge output, LVDEP and RVDEP. The responses to DMN stimulation were abolished by bilateral vagotomy. The responses to NA stimulation were abolished by cardiac pacing, the responses were secondary to bradycardia. The data suggest cardiac vagal preganglionic somata are organized according to physiological function. Cell bodies of the DMN control VC while NA somata are involved in HR regulation. (Supported by NIH Grant HL08682.)


Previous studies have demonstrated that blockade of GABA receptors in nucleus ambiguus with microinjections of bicuculline in the cat increased central vagal tone to the heart (DiMicco et al.: Proceed International Congres of Pharmacol., p. 755, 1978). This effect was reversed by the GABA receptor antagonist muscimol. These data suggested the existence of a GABAergic synaptic mechanism for the regulation of central vagal tone. To determine the physiological importance of this GABAergic system, the effects of drugs that modify CNS GABAergic synaptic transmission were tested on reflex-induced vagal bradycardia produced by activation of baroreceptors with i.v. bolus injections of phenylephrine. Chololamine-asesthetized cats were used and all drugs were administered into the fourth cerebroventricle. Administration of the GABA receptor antagonist muscimol (0.5 to 12.5 µg) to six cats produced approximately 85% of the reflex-induced vagal bradycardia produced by phenylephrine. This blockade was not due to antagonism of the presynaptic response of phenylephrine. Once blockade was present, administration of the GABA receptor antagonist bicuculline (25-30 µg) restored the reflex vagal bradycardia. This occurred without alteration in the phenylephrine-induced pressor response. Administration of bicuculline alone (1-5 µg) enhanced reflex-induced vagal bradycardia. These results suggest that a CNS GABAergic synaptic comprise part of the reflex vagal pathway in the cat.

Classically conditioned heart rate change in the pigeon has both vagal and sympathetic components. As part of a comprehensive effort to describe the discharge characteristics of motoneurons mediating this response, the activity of vagal cardiac neurons was recorded in trained animals. As previously reported (Gold and Cohen, 1979, 1980), a maintained decrease in discharge during the conditioned stimulus. This report now describes the discharge properties of such neurons during acquisition of the conditioned heart rate change.

Simple cell activity was recorded from the intermediate rostrocaudal (cardiac) zone of the right dorsal motor nucleus in immobilized, artificially ventilated pigeons. Units were identified as cardiac efferents by previously established criteria based upon antidromic activation and conduction velocity (Brain Res., 147, 79-90). Thirteen animals were given 40 trials of conditioning training where a 6-sec light presentation was immediately followed by foot-shock. Ten sensitization control animals received 40 unpaired stimuli. The light initially evoked a small cardiovascular response that was differentially affected by training (p<.02), being enhanced by conditioning and attenuated by sensitization.

Regarding neuronal activity, the groups did not differ with respect to conditioned discharge, and such discharge did not change significantly over training. The initial light presentation elicited a decrease in discharge that was most prominent during the initial 500 msec (phasic period) but persisted throughout the remaining 5500 msec of the light period (tonic period). In the conditioning group, a cell's phasic discharge increased significantly over training. The tonic decrease in discharge increased from 27% to 46%. In contrast, in the sensitization group, the phasic decrease in discharge was either not changed or decreased from 35% to 18% and the tonic response from 33% to 8%. Thus, the groups changed differentially (p<.001). Further analysis indicated that the latency of the decrease in discharge was 120-160 msec for both groups early in training. However, in the conditioning group this latency shortened to less than 100 msec, while in the sensitization group it increased to almost 200 msec.

These results clearly indicate vagal involvement in conditioned cardiovascular. Moreover, the vagal contribution is synergistic with that of the sympathetic cardiac output which shows phasic and tonic increases in discharge during conditioned stimulus presentation. (Supported by NSF grant BMS 75-20537 and NIH grants P01 NS14620 and T32 HG07284)


The Shy-Drager variant of Idiopathic Orthostatic Hypotension (IOH) is a progressive multisystem degenerative disorder which begins during middle age and is associated with autonomic and extrapyramidal system dysfunction. Recently, respiratory abnormalities such as persistent sleep impairment have been described, but the occurrence of laryngeal stridor has received little attention. We report three patients with Shy-Drager syndrome in whom laryngeal stridor developed. The clinical course and correlative neuropathological findings will be presented.

Laryngeal stridor presented a variable clinical picture in our patients. The first patient developed acute stridor with irregular respiration late during the course of his disease. The third patient developed interstitial stridor and irregular respiration.

To define the histological substrate of these respiratory abnormalities, lower cranial nerve nuclei and brain stem respiratory areas were examined in autopsy material obtained from these patients. Similar brainstem areas were examined in autopsies of 30 age-matched control patients. In the first patient, microscopical examination showed glialosis in the nucleus ambiguus (NA), trachea solitarius (TS), and nucleus tractus solitarius (NTS). Pathological examination of the second patient revealed gliosis in the NA, and glialosis, dystrophic axons and one area of phagocytosis in the TS and NTS. In the third patient, microscopic studies showed focal neuronal phagocytosis with a glial nodule in the NA, and diffuse gliosis in the medullary tegmentum. Postmortem examination of the second patient revealed abnormalities in the nucleus ambiguus (NA) and NTS. These observations suggest that laryngeal stridor is mediated by respiratory changes in the NA, and possibly NTS, in the Shy-Drager variant of IOH.

This work was supported by the NIH and the Dysautonomia Fdn. Inc. R.M.H. is the recipient of an Investigator Development Award NS 00383. I.B.B. is the recipient of the Irma T. Hirsch Career Scientist Award.)

MODIFICATION BY LIGHT HALOTHANE ANESTHESIA OF THE CARDIOVASCULAR CHANGES ASSOCIATED WITH REM SLEEP IN THE CAT. Richard E. Hall, Sandra C. Brown*, Wesley P. Norman*, Janette Dias Souza*, Maria L. C. Zanotto*, and Joel A. Hall, International Congress Pharmacol., p. 755, 1978), blockade of GABA receptor function by direct microinjection of bicuculline may correspond to REM sleep and not to an arousal response. Halothane anesthesia may have differential effects on central autonomic control and on sleep mechanisms and that the detection of EEG desynchronization during clinical light halothane anesthesia may correspond to REM sleep and not to an arousal response.

This work was supported by grants from NIH GMS 19797 and AHA-GLA 43716.

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In previous studies (Drobak et al., International Congress Pharmacol., p. 755, 1978), blockade of GABA receptor function by direct microinjection of bicuculline into the nucleus ambiguous (NA) produced a marked dose-related depression of heart rate which was mediated by the vagus. This effect was not obtained in other regions of the brainstem. The clinical course and correlative neuropathological findings will be presented.

Laryngeal stridor presented a variable clinical picture in our patients. The first patient developed rapid respiratory arrest with irregular respiration late during the course of his disease. The third patient developed interstitial stridor and irregular respiration.

To define the histological substrate of these respiratory abnormalities, lower cranial nerve nuclei and brain stem respiratory areas were examined in autopsy material obtained from these patients. Similar brainstem areas were examined in autopsies of 30 age-matched control patients. In the first patient, microscopical examination showed glialosis in the nucleus ambiguus (NA), trachea solitarius (TS), and nucleus tractus solitarius (NTS). Pathological examination of the second patient revealed gliosis in the NA, and glialosis, dystrophic axons and one area of phagocytosis in the TS and NTS. In the third patient, microscopic studies showed focal neuronal phagocytosis with a glial nodule in the NA, and diffuse gliosis in the medullary tegmentum. Postmortem examination of the second patient revealed abnormalities in the nucleus ambiguus (NA) and NTS. These observations suggest that laryngeal stridor is mediated by respiratory changes in the NA, and possibly NTS, in the Shy-Drager variant of IOH.

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CHANGES IN CENTRAL CHOLINERGIC NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Cinda J. Helle, Eric A. Muth and David M. Jacobsowitz. Lab. Clin. Sci. NIMH, Bethesda, MD 20205. Recent pharmacological studies have shown that CNS cholinergic mechanisms which may be involved in blood pressure control (Eur. J. Pharmacol. 361: 1997; Clin. Exp. Hypertension 1: 217, 1979) are altered in SHR (J. Hypertens. 1978). We are interested in investigating whether biochemical differences in cholinergic neurons of discrete brain nuclei exist between 4, 8 and 12 wk old SHR and age-matched matched Wistar Kyoto (WKY) control rats. The acetylcholine (ACh) concentration of the guinea pig is measured in hindbrain nuclei of 12 wk old SHR and WKY. Changes in ACh activity of several nuclei were observed in SHR of all three age groups. The greatest alteration (97% increase) was found in the CHAT activity of the locus coeruleus of 12 wk old SHR rats. A 315 increase in ACh content was also detected. No differences were found in this nucleus in 4 or 8 wk old SHR. Other nuclei known to be involved in blood pressure control which showed differences in CHAT activity in SHR compared to WKY rats were the paraventricular nucleus (36% decrease in 4 wk SHR), dorsomedial nucleus of the hypothalamus (193 and 235 decrease in 8 and 12 wk SHR, respectively), posterior hypothalamus (25% decrease in 12 wk SHR) and the nucleus reticularis gigantocellularis (28% and 21% decrease in 4 and 8 wk SHR, respectively). A significant increase in the CHAT activity of the anterior ventral thalamus was detected in 4 and 8 wk SHR.

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PHASE SHIFTING OF THE RESPIRATORY CYCLE PRODUCED BY ELECTRICAL STIMULATION OF THE MEDIAL PONTINE RETICULAR FORMATION. C.K. Knox, Laboratory of Neurophysiology, Department of Physiology, University of Minnesota, Minneapolis, MN 55455. The pathways and mechanisms underlying the effects of reticular stimulation on respiration are not well understood. It is possible that such stimulation affects respiratory centers either directly at the brainstem level, or, as Anderson and Sears (J. Physiol., London 209) have suggested, only indirectly at the spinal level via respiratory interneurons. We attempted to determine whether stimulation of the reticular formation in SHR that the locus coeruleus may sensitize the descending depressor systems in an attempt to combat the hypertension. In view of the widespread distribution of cholinergic neurons in the locus coeruleus, further exploration of this cholinergic neuroendocrine connection is warranted.

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PHASE SHIFTING OF THE RESPIRATORY CYCLE PRODUCED BY ELECTRICAL STIMULATION OF THE MEDIAL PONTINE RETICULAR FORMATION. C.K. Knox, Laboratory of Neurophysiology, Department of Physiology, University of Minnesota, Minneapolis, MN 55455. The pathways and mechanisms underlying the effects of reticular stimulation on respiration are not well understood. It is possible that such stimulation affects respiratory centers either directly at the brainstem level, or, as Anderson and Sears (J. Physiol., London 209) have suggested, only indirectly at the spinal level via respiratory interneurons. We attempted to determine whether stimulation of the reticular formation in SHR that the locus coeruleus may sensitize the descending depressor systems in an attempt to combat the hypertension. In view of the widespread distribution of cholinergic neurons in the locus coeruleus, further exploration of this cholinergic neuroendocrine connection is warranted.
ACTIVATION OF A PERIVENTRICULAR-PERIAQUEDUCTAL DESCENDING PATHWAY. Mark M. Knuepfer*, A.K. Johnson*, and M.J. Brody* (SPONSOR: also reported that descending neuronal tracts from AV3V stimulation. This study was designed to examine the effects of stimulation of the AV3V or the CG region elicited a frequency-dependent decrease in hindlimb vascular resistance and mesenteric and renal vasoconstriction. After bilateral adrenalectomy, the mesenteric and renal responses to both AV3V and CG stimulation were significantly attenuated. The hindlimb vasodilator response was attenuated by adrenalectomy for CG stimulation but not for AV3V stimulation. Intravenous infusion of epinephrine (1-2 µg/kg) resulted in a similar response to both sites. Stimulation of AV3V or CG region elicited changes similar to those produced by central stimulation of these two sites. After adrenalectomy, blockade (using phenolamine or guanethidine) further reduced responses to central stimulation. In summary, catecholamines released from the adrenal medulla appear to be a significant factor in CG-induced hindlimb vasodilation. We conclude that electrical activation of the descending pathway between AV3V and CG produces complex integrated regional vascular responses mediated by both efferent sympathetic innervation and circulating catecholamines released by central activation of the adrenal medulla. (Supported in part by USPHS Grants HL14388, GM70959, and 1-K02-NH00064.)

DO SYMPATHETIC NERVES CONTROL CARDIAC GROWTH? C. Lau*, R. Morgan*, D.L. Bareis* and T.A. Slotkin. Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, N.C. 27710 Development of the rat heart involves two growth phases: (1) in the first 2-3 weeks postnatal development rapid replication of myocardial cells occurs, associated with high rates of DNA synthesis, (2) thereafter, DNA synthesis and cell replication terminate and further growth occurs by cardiac hypertrophy involving RNA and protein synthesis. Previous studies have implicated involvement of sympathetic tone in both growth phases. Exposure of the neonatal heart to sympathomimetics has long been known to cause cardiac hypertrophy in the adult. Sympathomimetics have long been known to cause cardiac hypertrophy. In addition, the present hypothesis was tested by giving the neurotoxic agent α-methyltyrosine (α-MT) to produce complex integrated responses mediated by both efferent sympathetic innervation and circulating catecholamines released by central activation of the adrenal medulla. (Supported by USPHS Grants NS 14039)
FOUR CENTRAL NERVOUS SYSTEM SITES PROJECT TO THE PANCREAS.

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central nervous system sites projecting to the rat pancreas were identified and mapped using retrograde axonal transport of horseradish peroxidase (HRP). A total of 50µl of 30% (w/v) HRP (dilution Type VI) were injected into specific regions of each animal (n=12). After 48 hours, the animals were sacrificed, and their brains and spinal cords were processed for HRP with the tetramethylbenzidine (TMB) method of histochemistry. Serial 4µm sections of the tissue from the sacral cord to the caudal pole were saved and systematically analyzed for HRP reaction product.

Four well organized cell groups—two in the medulla and two in the spinal cord—were found to project to the pancreas. The largest number of cells with HRP reaction product formed a coherent group occupying the medial two-thirds of the dorsal motor nucleus (DMV) bilaterally at the rostral/caudal level of the area postrema. Another tightly clustered, smaller population in the spinal cord—were found to project to the pancreas. The A5 catecholamine cell group (Dahlstrom and Fuxe, '64) lies dorsal and lateral to the superior olive and medial to the laterally in the cervical cord (C2 to C3), and the other bilaterally in the lower thoracic region (approximately T5 to L1); the classic autonomic nucleus of the lateral horn. With the exception of a few isolated cells that were found along the intraocular medullary course of the vagal fibers (and that appeared to be "displaced" DMV neurons), no other CNS cell populations projecting to the pancreas were identified. In additional animals, HRP treatment of the proximal cut end of the cervical vagus high in the neck labeled the two pancreas cell groups in the medulla as well as the remainder of the neurons in the DMV and NA (virtually all palliallular to the nerve somas), but not the two columns in the spinal cord.

Supported by NIH Grant AM15511 and Career Development Award AM00563.

V. Leaungh* and J.L. Fowley* (SPON: Lynda Uphouse). Dept. Psychol., Yale University, New Haven, CT 06520.


The A5 catecholamine cell group (Dahlstrom and Fuxe, '64) lies dorsal and lateral to the superior olive and medial to the root of the VIIth cranial nerve. Two lines of anatomical evidence indicate that the A5 cell group projects to medulla and spinal vasomotor centers. First, stereotaxic injections of H amino acids were made in the A5 cell group in rats. As demonstrated by autoradiography, a spinal projection from this area descends via the dorsolateral funiculus to the intermediolateral and intercalated sympathetic cell groups of the thoracic and upper lumbar spinal cord. There are also descending projections to the medulla and paraventricular reticular formation, the medial and parvocellular regions of the solitary complex, and the dorsal motor nucleus of the vagus. After similar injections in animals pretreated with 6-hydroxydopamine (250 µg, intraventricularly), there was no evidence of a spinal projection. Second, we used the combined histofluorescence-retrograde cell labeling technique of Blessing et al. ('78) to show that the A5 catecholamine cells are retrogradely labeled after injections of HRP into the Ti-T2 spinal cord. Control injections of HRP into the femoral vein or subarachnoid space at the Ti levels failed to label the A5 neurons. To provide further evidence, we studied the retrograde labeling pattern in the A5 cell group of rats that had received 6-hydroxydopamine injections in the lateral ventricle and this treatment abolished virtually all retrograde cell labeling in this area from HRP injections in the spinal cord.

These observations indicate that the A5 cell group is one source of the preganglionic input to vasomotor centers of the medulla and spinal cord.

Supported by USPHS grant NS 12751 and American Heart Association grant 77-797.

The baroreceptor reflex in a-cholinergic anesthetized cats was stimulated by (1) complete carotid occlusion and electric stimulation of the (2) right and (3) left vagus nerves. Chlorpromazine (CPZ) was administered in cumulative doses of 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.50, 1.0, 2.0, and 4.0 mg/kg over an approximately 4-hour time period. CPZ produced a highly significant change in blood pressure whose duration was dose-dependent. CPZ also resulted in a more variable blood pressure response to carotid occlusion (p<0.005) and to right vagus stimulation (p<0.005) and to vagus stimulation over time. The mean blood pressure response to each of the 3 test stimuli was not affected in a dose related fashion. Also, the latency between carotid occlusion and maximum increase in blood pressure was not significantly affected by CPZ.

However, in nearly all animals CPZ (n=8) produced a more variable basal blood pressure (p<0.005) than in control animals (n=8). In some cases CPZ caused a repetitive oscillation in blood pressure at frequencies of 1-2/minute, whose duration was dose-dependent. CPZ also resulted in a more variable blood pressure response to carotid occlusion (p<0.005) and to right vagus stimulation (p<0.005) and to vagus stimulation over time. The mean blood pressure response to each of the 3 test stimuli was not significantly affected by CPZ. These effects are shown below.

In three additional cats, each receiving single rather than cumulative doses of CPZ, the drug produced marked decreases in blood pressure. In two of these, the blood pressure returned toward the pre-drug value within 30 minutes.

The results show that although mean blood pressure and the baroreceptor reflex are not depressed by CPZ, the drug results in more variable blood pressure control, perhaps by de-stabilization of the baroreceptor reflex.

Supported by NIH Grant AM15511 and Career Development Award AM00563.


An experiment is described which addresses the potential for non-invasive assessment of vagal tone to the heart. Previous research has demonstrated that respiratory sinus arrhythmia (RSA) is mediated primarily through the vagus and that quantitative evaluation of the magnitude of RSA provides an accurate estimate of vagal tone in the anesthetized preparation. This research focuses on the validation of a statistical method of detecting shifts in vagal tone based on the heart rate variance (HRV) which is associated with respiratory influences. In the preparation used in this study, respiratory influences are the primary source of HRV. In unanesthetized preparations behavioral and physiological influences may affect the variability of heart rate. Therefore, it is necessary to partition the respiratory influence on HRV from the total HRV. Spectral analysis, applied to heart rate, can decompose the HRV into its constituent frequencies. Thus, the HRV distributed across the respiratory frequencies may be used to quantify a component of HRV which may be sensitive to shifts in the vagal tone to the heart.

To assess this method, the aortic depressor nerve (ADN) of anesthetized rabbits was stimulated for 30 seconds (.1 msc pulse duration, 100 pps, .05-2mA). The stimulation, independent of current level, produced a reflex bradycardia. Since stimulation of ADN increases vagal efferent activity to the heart, spectral analysis was applied before and during stimulation. ANM stimulation increased HRV associated with the respiratory frequencies in all animals at all currents tested. This supports the contention that the spectral estimate of this component of HRV is sensitive to increases in vagal tone to the heart.

However, for high current levels this increase in HRV was not significan in a dose related fashion. In some cases CPZ (n=8) produced a more variable basal blood pressure (p<0.005) than in control animals (n=8). In some cases CPZ caused a repetitive oscillation in blood pressure at frequencies of 1-2/minute, whose duration was dose-dependent. CPZ also resulted in a more variable blood pressure response to carotid occlusion (p<0.005) and to right vagus stimulation (p<0.005) and to vagus stimulation over time. The mean blood pressure response to each of the 3 test stimuli was not significantly affected by CPZ. These effects are shown below.

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Supported by NIH Grant AM15511 and Career Development Award AM00563.
S.H. Mytilineou and Maria C. Papaconstantinou* . Dept. of Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

In later experiments we found that three second stimulation of the perifornical posterior hypothalamus (PPFH) produces activity in the splanchnic nerve, proximal to the celiac ganglion (PCSA) which rises rapidly during the first, and declines steadily during the second and third second of stimulation. The decline represents inhibitory influences of the baroreceptor reflex activated by the evoked rise in blood pressure and can be blocked by sinoaortic denervation, or by preventing the pressure rise with hexamethonium (HEX).

In an earlier work we have applied a similar analysis to the PPFH response to stimulation of the dorsolateral spinal cord (DLC) at C1. In 250-300 gm. male rats (SHR and WKY), anesthetized with 100mg/kg alpha-chloralose, paralyzed with Flaxedil and artificially respired, a portion of the greater splanchnic nerve, proximal to the celiac ganglion was placed on bipolar platinum hooks. Activity was filtered(300-3K) and digitally integrated.

In most preparations DLC stimulation produced a time course of PCSA similar to that evoked from PPFH; a decline in PCSA occurring during the 2nd and 3rd seconds of DLC stimulation as blood pressure rose. When HEX (25 mg/kg) was given i.v., resting pressure fell to about 70 mm Hg, and evoked pressure changes from PPFH or DLC were eliminated. Evoked activity in the first second of PPFH or DLC stimulation was relatively unchanged while activity in the 2nd and 3rd seconds decreased as compared to pre-HEX responses. Since evoked activity in the 2nd and 3rd seconds of DLC stimulation remained after ipsilateral hemisection of the cord medial to C1, the decline in PCSA in the 2nd and 3rd seconds in pre-HEX responses probably is due to baroreceptor reflex influences acting at the spinal level.

In some preparations, HEX produced a fall in spontaneous PCSA and the entire response to DLC stimulation was reduced by 30-70%. Pre-HEX PCSA response levels could be restored by increasing the intensity of DLC stimulation. We conclude that time course analysis can be applied to PCSA responses obtained with DLC stimulation, allowing measurement of both sympatho-excitation and reflex inhibition acting at the spinal level.

CHANGES IN THE ORGANIZATION OF THE MICTURITION REFLEX PATHWAY (MRP) IN THE CAT FOLLOWING TRANSECTION OF THE SPINAL CORD.

Previous studies showed that micturition in cats with an intact neuraxis was dependent on a sacral reflex. This report describes the changes in the MRP in chronic spinal cats 1 to 14 weeks after spinal cord transection at T13. Reflexes were recorded postganglionic fibers from the bladder in response to stimulation of afferents in the pelvic nerve. In chronic spinal cats, reflexes occurred at long latency (150-180 msec) and only at stimulus intensities which activated C-fiber afferents. The reflex, which was usually sufficiently intense to evoke bladder contractions, could be elicited when the bladder was distended with fluid or completely empty. It occurred only ipsilaterally to the site of stimulation and was not affected by bilateral transection of the hypogastric nerves or the sympathetic chain. The reflex was abolished by mechanical stimulation of the anal canal.

In cats with an intact neuraxis reflexes were detected at shorter latencies (95 to 140 msec) contralateral as well as ipsilateral to the site of stimulation at stimulus intensities which activated A6 fibers. In 60% of these animals (7 of 11 experiments) a longer latency reflex (180-200 msec) was also observed when the stimulus intensity was increased above the threshold for C-fiber afferents. The late reflex was also observed contralaterally as well as ipsilaterally to the site of stimulation. Neither the early nor the late reflex could be elicited when the bladder was empty. Neither reflex was affected by bilateral transection of hypogastric nerves, but both reflexes were abolished by spinal section at T10. 24 to 48 hours after cord transection at T13 a very weak reflex which corresponded in latency and threshold to the C-fiber reflex could be elicited with trains of stimuli 11 to 15 pulses in the pelvic nerve.

We conclude that in chronic spinal cats the MRP undergoes a reorganization which is characterized by: (1) functional disconnection of the A-fiber component of the peripheral afferent limb, and (2) a change in the central pathway from a primarily supraspinal reflex distributed bilaterally to a spinal reflex distributed ipsilaterally. The reemergence of a strong spinal reflex one week after cord transaction accounts for the development of automatic micturition in chronic spinal animals. (Supported by NIH Grant 70923-11, and an Overseas Research Fellowship from the Medical Research Council of New Zealand to R.J.M.)
AUTONOMIC FUNCTION

161 RELATIONSHIP OF PLASMA EPINEPHRINE AND NOREPINEPHRINE LEVELS IN RECUMBENT HUMANS. Benjamin H. Natelson and Barry E. Levin. VA Medical Center and Dept. of Neurosciences, CMDN-New Jersey Medical School, East Orange, NJ 07018.

If changes in epinephrine (E) and norepinephrine (NE) reflect some common central mechanism of autonomic function, plasma levels of the 2 catecholamines should relate well with each other under a variety of different conditions. To examine this issue, whole blood plasma samples were collected from healthy subjects during a series of social interactions. Plasma norepinephrine (NE) and epinephrine by a radioenzymatic method, NE and E levels in each subject were measured daily and found to vary only slightly over a 5 day period following surgery (range about 1.5 h r ; and (c) humans can be divided into 2 groups based on the presence or absence of sleep among the 2 groups. The presence of the male or female second animal. The presence of the male or female second animal. The presence of the male or female second animal. The presence of the male or female second animal. Subject arrived in the laboratory 1 hr before the start of sampling while subjects in the second group slept overnight in the laboratory. Deproteinized plasma was frozen and subsequently assayed for norepinephrine and epinephrine by a radioenzymatic method. NE and E levels in the first group both decreased in a similar fashion over time (median r of log transformed data = .75, p < .01). Because this pattern of relatively high early values was seen in subjects studied in both the morning and afternoon, we conclude that the downward tendency was related to our experimental procedure. This was verified in our second group of subjects who showed no consistent changes in NE and E over time. To further study the relation between NE and E, we determined if by computing deviations from a linear regression, the difference between the two subject's log-transformed NE or E values. Correlations between NE and E residuals fell in 2 groups: those with a poor relation (r ranging from .10 to .43, n = 6), and those with a strong relation (r ranging from .60 to .86, p < .01, n = 6). Time series statistics were then applied to both NE and E residuals in the group studied for 4 hr. Peak power ranged from frequencies of 34–188 min (median = 107 min) for NE and from frequencies of 59–188 min (median = 94 min) for E. Our conclusions from these results are that (a) some common process is responsible for tonic stimulation of NE and NE because of the invariable downward slope in both substances over time, probably related to our experimental design; (b) superimposed on this tonic factor are phasic fluctuations in both neuro-humors with a median ultradian rhythm of about 1.3 hr; and (c) humans can be divided into 2 groups based on whether or not they show phasic rhythms of NE or E correlating highly. Thus NE and E correlate well with each other in some but not all conditions; this suggests that central control of release of these neurohormones may be due to activation of anatomically discrete but interrelated areas.

162 MULTIPLE SPINAL PATHWAYS DESCENDING FROM THE FASTIGIAL NUCLEUS. Carl A. Ohta, Robert D. Foreman and Kenneth J. Dorner. Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190.

Electrical stimulation of the fastigial nucleus (FN) in the cervical cord induces in dogs changes in the vegetative and sympathetic efferent. FN-evoked potentials are present in pragnagnic fibers of the Tg white ramus communicans and the splanchnic nerve. FN stimulation evokes a marked bradycardia response, an initial tachycardia which is followed by baroreceptors, and an increase in plasma renin activity. The object of this study was to determine the effects of spinal lesions on evoked potentials in the splanchnic nerve and on the pressor response. The right or left FN was located with a stimulating electrode in dogs anesthetized with chloralose (115 mg/kg). The splanchnic nerve was cut and evoked potentials were recorded from multifiber units in the central end. Lesions were made successively in different segments of the spinal cord. The fastigial pressor response was unaffected by T1 spinal transection, moderately attenuated by lesions placed in the T5, T5 dorsalateral funiculi and T5 spinal transection, and abolished by Cg transection. This indicates that sympathetic efferents affecting blood pressure leave the spinal cord primarily in the upper thoracic segments (T1 to T5). FN stimulation also increased the activity of multifiber units in the splanchnic nerve. At the end of pressor stimulation, the spontaneous discharge of some splanchnic units was inhibited during maximal and declining pressure presumably via the baroreflex. This inhibition was attenuated or abolished by transection of the T6 cord. The splanchnic potentials were relatively unaffected by T1 spinal transection although sometimes the spontaneous activity and evoked response were abolished but with persistence in the conduction latency. The effect of subsequent spinal lesions on the FN-evoked response indicate multiple pathways of splanchnic preganglionic fibers. These units were unimpaired by spinal lesions suggesting that the preganglionic fibers leave the cord in the upper white rami then descend in the sympathetic chain. The evoked response involves only a few units. Thus the spinal lesions indicating that splanchnic fibers also leave the lower thoracic cord. Still other multifiber units were activated by mid-thoracic lesions suggesting that fibers exit from the upper and lower thoracic segments. All evoked responses were abolished after Cg transection. In conclusion the fastigial pressor response is conveyed primarily by fibers departing from the upper thoracic cord. Splanchnic fibers may depart from various segments of the thoracic spinal cord. Splanchnic multifiber units were excited by FN stimulation and sometimes inhibited by a convergent descending baroreceptor pathway. (Supported by NIH grant HL05670 and the Oklahora Heart Association).

163 SOCIAL INTERACTION, STRESS AND CATECHOLAMINES IN RATS. S. PASHKO, K. DETURCK* and W. H. VOGEL. Thomas Jefferson University, Department of Pharmacology, Philadelphia, PA 19107 USA.

Male wistar rats were catheterized in the jugular vein and the silicon tubing protected with a steel spring which was supported by a swivel pulley and a counterweight. The animal could move relatively freely. Drugs that modify CNS cholinergic synaptic transmission were administered into the fourth ventricle. Propantheline (0.2–0.5 mg), a muscarinic receptor blocking agent, administered to six animals to five animals exhibiting reflex vagal bradycardia (e.g., Rozear et al.: Int. J. Neuropharmacol. 7: 1, 1968), experiments using chloralose-anesthetized rats, were performed whereby baroreceptors were activated with i.v. bolus injections of phenylephrine and the effects of drugs that modify CNS cholinergic transmission were observed. Drugs tested were propantheline, neostigmine, and hexamethonium, and were all administered into the fourth cervical ventricle. FN-evoked potentials (i.e., a) were recorded from the splanchnic nerve. At the end of pressor stimulation, the splanchnic nerve and on the pressor response. The right or left FN was located with a stimulating electrode in dogs anesthetized with chloralose (115 mg/kg). The splanchnic nerve was cut and evoked potentials were recorded from multifiber units in the central end. Lesions were made successively in different segments of the spinal cord. The fastigial pressor response was unaffected by T1 spinal transection, moderately attenuated by lesions placed in the T5, T5 dorsalateral funiculi and T5 spinal transection, and abolished by Cg transection. This indicates that sympathetic efferents affecting blood pressure leave the spinal cord primarily in the upper thoracic segments (T1 to T5). FN stimulation also increased the activity of multifiber units in the splanchnic nerve. At the end of pressor stimulation, the spontaneous discharge of some splanchnic units was inhibited during maximal and declining pressure presumably via the baroreflex. This inhibition was attenuated or abolished by transection of the T6 cord. The splanchnic potentials were relatively unaffected by T1 spinal transection although sometimes the spontaneous activity and evoked response were abolished but with persistence in the conduction latency. The effect of subsequent spinal lesions on the FN-evoked response indicate multiple pathways of splanchnic preganglionic fibers. These units were unimpaired by spinal lesions suggesting that the preganglionic fibers leave the cord in the upper white rami then descend in the sympathetic chain. The evoked response involves only a few units. Thus the spinal lesions indicating that splanchnic fibers also leave the lower thoracic cord. Still other multifiber units were activated by mid-thoracic lesions suggesting that fibers exit from the upper and lower thoracic segments. All evoked responses were abolished after Cg transection. In conclusion the fastigial pressor response is conveyed primarily by fibers departing from the upper thoracic cord. Splanchnic fibers may depart from various segments of the thoracic spinal cord. Splanchnic multifiber units were excited by FN stimulation and sometimes inhibited by a convergent descending baroreceptor pathway. (Supported by NIH grant HL05670 and the Oklahora Heart Association).


To confirm earlier findings of others that a CNS cholinergic mechanism is involved in reflex-induced vagal bradycardia (i.e., a) in the dog (Hegarty et al. Pharmacol. 2: 1, 1968), experiments using chloralose-anesthetized cats, were performed whereby baroreceptors were activated with i.v. bolus injections of phenylephrine and the effects of drugs that modify CNS cholinergic transmission were observed. Drugs tested were propantheline, neostigmine, and hexamethonium, and were all administered into the fourth cervical cord ventricles. FN-evoked potentials (i.e., a) were recorded from the splanchnic nerve. At the end of pressor stimulation, the splanchnic nerve and on the pressor response. The right or left FN was located with a stimulating electrode in dogs anesthetized with chloralose (115 mg/kg). The splanchnic nerve was cut and evoked potentials were recorded from multifiber units in the central end. Lesions were made successively in different segments of the spinal cord. The fastigial pressor response was unaffected by T1 spinal transection, moderately attenuated by lesions placed in the T5, T5 dorsalateral funiculi and T5 spinal transection, and abolished by Cg transection. This indicates that sympathetic efferents affecting blood pressure leave the spinal cord primarily in the upper thoracic segments (T1 to T5). FN stimulation also increased the activity of multifiber units in the splanchnic nerve. At the end of pressor stimulation, the spontaneous discharge of some splanchnic units was inhibited during maximal and declining pressure presumably via the baroreflex. This inhibition was attenuated or abolished by transection of the T6 cord. The splanchnic potentials were relatively unaffected by T1 spinal transection although sometimes the spontaneous activity and evoked response were abolished but with persistence in the conduction latency. The effect of subsequent spinal lesions on the FN-evoked response indicate multiple pathways of splanchnic preganglionic fibers. These units were unimpaired by spinal lesions suggesting that the preganglionic fibers leave the cord in the upper white rami then descend in the sympathetic chain. The evoked response involves only a few units. Thus the spinal lesions indicating that splanchnic fibers also leave the lower thoracic cord. Still other multifiber units were activated by mid-thoracic lesions suggesting that fibers exit from the upper and lower thoracic segments. All evoked responses were abolished after Cg transection. In conclusion the fastigial pressor response is conveyed primarily by fibers departing from the upper thoracic cord. Splanchnic fibers may depart from various segments of the thoracic spinal cord. Splanchnic multifiber units were excited by FN stimulation and sometimes inhibited by a convergent descending baroreceptor pathway. (Supported by NIH grant HL05670 and the Oklahora Heart Association).
166 MEDIAL POSTERIOR HYPOTHALAMIC LESIONS INHIBIT HYPERTENSION IN SPROUTINGLY HYPERTENSIVE RATS. Elinor Zilkey*, Kazuo Takeda, and Ruben Bunag. Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, Kansas 66103. Although evidence exists for autonomic hyperactivity and endothelial dysfunction in the etiology of the hypertension of the medial hypothalamic rat (SRH), the exact role of the nervous system is unknown. Bilateral lesions (stainless steel electrodes, 0.2 x 0.5 mm exposed length, 2 mA, 0.5 sec DC amodal current, rectal cathode) or sham operations (electrode placement without current passage) were made in 55-day-old female SHR and their normotensive Kyoto Wistar-derived counterparts (WKY, both from Charles River Laboratories). Systolic blood pressure, heart rate, and body weight were recorded weekly up to 16 weeks of age (9 weeks after lesion). The rats were housed in a limited access room (lights on 0700-1900 h) where measurements were also made (AM) using a tail cuff method with preheating and a 25 mm cuff. Blood pressure did not change in WKY but was significantly lower in lesioned than in sham operated SHR throughout the experiment. Heart rate was reduced in both WKY and SHR. Although an initial weight loss in lesioned SHR was followed by normal weekly gain, body weights remained lower than those of controls.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Blood pressure</th>
<th>Heart rate</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY sham (6)</td>
<td>108±11</td>
<td>43±13</td>
<td>20±26</td>
</tr>
<tr>
<td>WKY lesion (6)</td>
<td>107±5</td>
<td>35±11*</td>
<td>20±16</td>
</tr>
<tr>
<td>SHR sham (9)</td>
<td>167±4</td>
<td>45±8</td>
<td>17±4</td>
</tr>
<tr>
<td>SHR lesion (9)</td>
<td>127±7</td>
<td>38±13*</td>
<td>15±4*</td>
</tr>
</tbody>
</table>

When sympathetic nerve activity (splanchnic nerve) was recorded in sham and lesioned SHR (urethane anesthesia) 10 weeks after lesion, basal nerve activity and changes in blood pressure and nerve activity in response to anterior hypothalamic stimulation were significantly reduced in lesioned SHR. Our results suggest that hypothalamic lesions inhibit the rapid blood pressure elevation that normally occurs in SHR at the age of about 5-12 weeks and that this inhibition is possibly due to reduced sympathetic output. (Supported by NIH Grant HL 14560.)

167 DESCENDING CONNECTIONS FROM THE BRAIN TO THE SPINAL CORD IN THE RAT, AS STUDIED WITH HORSE RADISH Peroxidase. Christopher Ronan, David A. Ruggiero* and Donald J. Reis. Lab. of Neuroblol., Dept. Neurol., Cornell Univ. Med. College, New York, NY 10021. With the objective of defining descending central autonomic pathways in the rat, we have injected horseradish peroxidase (HRP) into thoracic segments of the spinal cord and compared the distribution of retrogradely transported enzyme with that following injections at other levels. HRP (0.2 microliters of 10% saline solution) was unilaterally injected into cervical, upper or lower thoracic, lumbar, or sacral levels of rat spinal cord. Following 48-hr survival, brains were sectioned and the HRP reaction product was localized in the solitary complex, the spinal trigeminal nucleus, the reticular nucleus, portions of the nucleus gigantocellularis (pons), the periventricular area, entopeduncular and paraventricular nuclei, lateral hypothalamic and cerebral cortex. Labeled neurons in the locus coeruleus were restricted primarily to ventral regions, suggesting a topographic organization of this nucleus's efferent connections. The existence of labeled cells in the paraventricular and lateral hypothalamus confirms the previous report of direct hypothalamic-spinal connections in the rat (Saper et al., Brain Res. 117, 305). In contrast to other species, few if any neurons from the spinal nucleus and tectum project to spinal cord in the rat. A striking connection (previously undescribed) from PPM to the PML, especially the cervical and lumbar enlargements, appears significant when related to the importance of PPM as a terminus of pallidal and precentral cortical efferents, and may represent a direct extrapyramidal cholinergic signal for stimuli from the basal ganglia to the spinal cord. Projects predominantly to thoracic levels of the cord were observed from the lateral hypothalamus, the paraventricular nucleus, reticular nucleus, portions of the nucleus gigantocellularis (pons), and the nucleus paraventricularis, supporting physiological evidence that these nuclei may be of particular importance in central autonomic regulation.

(Supported by NIMH grants HL 18974, HL 07379, and the N.Y. Heart Association.)
171 VAGAL CARDIAC INNERVATION: COMPARATIVE CONTRIBUTIONS OF THE DMN and NA both contribute to the cardiac innervation in the cat and the rat. There are differences in the cardiac termination of each nucleus, the DMN of the rat, for example, predominantly and NA both contribute to the cardiac innervation in the cat and the rat. We sought to establish different projections to this region by use of a modified horseradish peroxidase (HRP) technique (Ruggiero et al., 1977). HRP was injected into sites of the DMN and NGC, and labeled axons were followed from low intensity electrical stimulation elicited an appropriate cardiovascular response. In general, there was a differentiation of different projections into these structures; several projections, however, overlap both nuclei. Afferents to HRP derive predominantly from dorsolateral frontal cortex (FC), ventromedial area incerta, ventral central grey (CG), nucleus of the Kölliker-Fuse nucleus, solitary complex and most lamina of CSC. Neurons of the DMN and the NA may project to the heart as distinct subsets of cardiac afferent feedback, possibly via the n. tractus solitarius (NTS). The reduced phasic modulation of NPBM neuronal activity during QS may result from forebrain inhibitory influence. The increase in CA synthesis in sodium sensitive rats after high sodium diet, coincides with clinical hypertension. These results suggest that adrenomedullary catecholamines may be implicated in the development and maintenance of the hypertension in the Dahl rats.

170 ADRENAL CATECHOLAMINES IN THE SODIUM SENSITIVE, HYPERTENSIVE RATS (DAH RATS). J.M. Saavedra, R. Del Carman*, R. McCarty*, V. Welse*, and J. Iwai* (SPoN: G. Gilad) National Institute of Mental Health, Bethesda, MD 20892. The effects of high (8%) and low (0.4%) sodium diet in adrenal catecholamines (CA) have been studied in the sodium resistant and the sodium sensitive Dahl Rats. When kept under low sodium diet, the sodium sensitive rats have increased dopamine-beta-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) activities with respect to the sodium resistant animals, indicating an increased synthesis of CA. The effects of a high sodium diet were opposite in both strains. High sodium resulted in the synthesis of CA in the sodium resistant rats (decreased tyrosine hydroxylase (TH), DBH and CA). In contrast, the sodium sensitive rats respond to increased TH, PNMT and CA.

172 CARDIAC-RELATED DISCHARGE OF NEURONS IN THE MEDIALL PARAVERTEBRAL NUCLEUS OF THE CAT DURING SLEEP-WAKING STATES. Gary C. Sieck* and Ronald M. Harper (SPoN: Thelma Estrin). Department of Anatomy and Brain Research Institute, University of California School of Medicine, Los Angeles, CA 90024. The discharge of single neurons in the nucleus parabrachialis medialis (NPBM) was examined in anesthetized, unrestrained cats across sleep-waking states. Adult cats were anesthetized with sodium pentobarbital, and electrodes were implanted for monitoring EEG, EOG, EKG, lateral geniculate nucleus EEG, and dorsal hippocampal EEG. A bundle of 10 fine-wire electrodes (62 μ) insulated microdure, together with a miniature microdure, were implanted stereotaxically in the NPBM. Recordings were initiated in a total of six cats after they had recovered from these surgical procedures. Sleep-waking states were assessed using standard physiological and behavioral measures. Cardiac relations in NPBM neuronal activity during each sleep-waking state were determined by calculating cross-correlation functions between neuronal discharge and a reference event at the peak of the 'R' wave of the EKG. Different bin widths were used in the cross-correlations to assess phase relationships between the neuronal spike train and the EKG reference. Cardiac relations were observed in approximately 25% of the 60 NPBM neurons examined. The extent of the phasic cardiac modulation of NPBM neuronal activity varied between cells and sometimes across states for individual cells. In some NPBM neurons, cardiac relationships in discharge appeared during only 1 or 2 sleep-waking states. Generally, cardiac relationships in neuronal activity during quiet sleep (QS) were absent or reduced compared to waking (W) and REM sleep states. Most NPBM neurons that showed cardiac relations increased their discharge 0-50 msec after the peak of the 'R' wave. Different bin widths were used in the cross-correlation functions to assess phase relationships between the neuronal spike train and the EKG reference.

Muscimol (M) a GABA receptor agonist, acts centrally to decrease blood pressure (BP), heart rate (HR) and sympathetic nerve splanchnic outflow (Antonaccio et al.: J. Pharmacol. Exp. Ther. 26: 283, 1977). The present study defines the areas within the brain that are mediating the hypotensive effects of M. In chloralose anesthetized cats, M was perfused into the lateral ventricle to decrease BP, HR and sympathetic nerve activity (SNA). To differentiate the sites of action in the medullary region, M was administered into either the caudal or anterior region of the 4th ventricle. Administration of M (0.003-0.03 μg/kg/min) for 30 min ACV produced dose related decreases in BP, HR and SNA with the highest dose of M producing maximum reductions of 40%, 30% and 30%, respectively. When the largest dose of M was prevented from reaching the 4th ventricle, a reduction in BP and HR of 15% occurred. Administration of M (0.03 μg/kg/min) into the caudal region of the 4th ventricle failed to alter BP, HR or SNA. However, administration of clonidine (1.0 μg/kg/min) for 10 min, an α agonist, to the same area of the medulla led to a 30% fall in BP and 20% fall in HR. In contrast to the lack of effect of M in the caudal region of the medulla, perfusion of M (0.03 μg/kg/min) into the anterior region of the 4th ventricle produced maximum decreases in BP and HR. Pretreatment with the GABA receptor antagonist bicuculline methiodide administered into the cerebral aqueduct in a dose which produced virtually no change in BP, HR or SNA (0.5 μg/kg/min) blocked or markedly reduced the effects of M administered ICV. These results indicate that the major sites mediating the hypotensive actions of M are localized in the anterior region of the medulla and/or on the arterial surface of the medulla. The actions of M were blocked by pretreatment with the GABA receptor antagonist bicuculline suggesting the hypotensive effects are mediated by activation of GABA receptors. Furthermore, the medullary site of action of M is marked by the presence of clonidine receptors which lowers BP and HR by activating central α adrenergic receptors. There appears to be a supramedullary site which contributes to the hypotensive effects of M.

A POSSIBLE RULE OF OPIATE RECEPTORS IN THE PRESSOR RESPONSE TO ANGIOTENSIN II (AI1) INFUSED VIA THE Vertebral ArTERies (VA) when compared to the intravenous (i.v.) route of administration was attributed to the depressant effects of pentobarbital anesthesia. A centrally mediated pressor response (PR), however, was unmasked when chloralose anesthesia was used in combination with morphine. The recent demonstration of opiate receptors in the same regions of the medulla where all α acts to facilitate central sympathetic activity caused us to wonder whether the action of morphine in facilitating the hypotensive actions of M are localized in the anterior region of the medulla and/or on the arterial surface of the medulla. The actions of M were blocked by pretreatment with the GABA receptor antagonist bicuculline suggesting the hypotensive effects are mediated by activation of GABA receptors. Furthermore, the medullary site of action of M is marked by the presence of clonidine receptors which lowers BP and HR by activating central α adrenergic receptors. There appears to be a supramedullary site which contributes to the hypotensive effects of M.

It has been observed that the magnitude of respiratory related sinus arrhythmia (RSA) in cats varies according to sleep/waking state (Baust and Bohnert, Exp. Br. Res. 7:169, 1969). Meaningful quantification of RSA has been difficult, however, because of the complex behavior of the heart rate in response to multiple reflex and non-reflex influences. In order to determine the magnitude and character of respiratory related RSA, we have undertaken the following: we have performed spectral analysis and coherence calculations on cardiac rate variations and respiratory activity. Five adult cats were implanted with cortical EEG, EOG, LCN, hippocampal EEG, and ECG electrodes for chronic recording. Respiratory movements were monitored with a thoracic strain gauge, and continuous polygraphic recordings from behavior were obtained during multiple sleep/waking periods. Quiet wakefulness (AW), quiet sleep (QS), and active sleep (REM) were identified by standard polygraphic criteria. Extent of RSA (Xr; log spectral amplitude of heart rate as a function of RSA) and coherence of heart rate variation with respiration (Cr) were calculated on a minute-by-minute basis from fast Fourier transforms of respiratory activity and interpolated heart rate intervals. Paired T-tests were used to assess differences in RSA between states. Xr and Cr were greatest in QS in all cats. Although Xr during QS showed considerable variation between cats, Cr was very high (approaching 0.9) for every QS segment examined. In contrast to previous observations, we found that respiratory related sinus arrhythmia decreases with increasing REM, with RSA magnitude being significantly less than those of QS. Extent and coherence were also significantly less in AW than in QS. Sleep thus exerts a powerful effect on respiratory modulation of cardiac rate variation. Quiet sleep represents a state wherein the strongest coupling occurs between these two physiological variables.

(Supported by USPHS ROI HL-22418-02. Computing assistance was provided by the Data Processing Laboratory of the Brain Research Institute, supported by grant NS 02501 from USPHS.)


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EVIDENCE FOR THE PRESENCE OF A TONICALLY ACTIVE FOREBRAIN GABA SYSTEM INFLUENCING CENTRAL SYMPATHETIC OUTFLOW IN CATS.


Bicuculline administered into the lateral cerebroventricle of chloralose anesthetized cats produces increases in arterial pressure and heart rate. To determine whether this effect is localized in forebrain areas, bicuculline in doses of 1.5 and 25 ug was administered into either the third and lateral ventricles with cannulation of the cerebral aqueduct or into the fourth ventricle of vagotomized cats. Administration into the third and lateral ventricles resulted in dose-dependent increases in pressure and rate, with the maximal response being 63 ± 11 mm Hg and 44 ± 9 beats/min., respectively. These same doses had no significant effect on heart rate and only a slight effect on pressure when administered into the fourth ventricle. Administration of the GABA receptor agonist, muscimol, into the third and lateral ventricle in a dose that had no effect alone on pressure and rate (10 ug) prevented the cardiovascular effects of bicuculline. In contrast, administration of the centrally active antihypertensive agent clonidine (31 ug) had no effect on bicuculline-induced increases in pressure and rate. In addition, muscimol (10 ug) when administered at the time of the peak pressure and rate responses of bicuculline restored these indices of cardiovascular function to normal. Clonidine (31 ug), however, administered in the same fashion had no effect. These results suggest that a tonically-active GABAergic system exists in the region of the forebrain and exerts inhibitory control over sympathetic activity influencing arterial pressure and heart rate.
AXONAL TRANSPORT
FROM THE DORSAL COLUMN NUCLEI TO THEIR TERMINAL TARGETS IN CATS.

column nuclei (DCN) in cats produce degeneration in the inferior dense labeling in 10, despite the fact that neurons at the 3H-pro samples, however, were taken from areas in DCN which are known to contain 10-projecting neurons and in which small 3H-pro injections still produce heavy labeling in 10. (2) 3H-pro-labeled molecules may be transported very rapidly out of the DCN, precluding their visualization at a 24 hr survival time. The same labeling pattern is seen, however, even at a 20 min survival time. (3) Since the method is used here for the visualization only of bound amino acids, it may be that 3H-pro is seen in DCN because it is taken up and transported from the perikaryon in its free form before being bound. It is generally observed, however, that no acids are incorporated into molecules within the perikaryon. These considerations suggest that the paucity of neuronal labeling by 3H-pro in DCN as seen in the present experiments does not reflect an artificial situation. If so, then some 3H-pro-labeled molecules must be transferred to 10 (as well as to other terminal targets) by a neuronal mechanism. One such mechanism might involve glial cells. Consistent with this suggestion is the fact that cells small enough to be glia are labeled in 10 when NPR is injected in 10. The glial cells and oligodendrocytes could be involved in this transfer mechanism. Most astrocytes and their processes are heavily labeled following 3H-pro injections along with some oligodendrocytes and cells. Astrocytes with very long processes are known to exist and the fibers which project to 10 are myelinated. Thus, it appears that molecules labeled by tritiated amino acids may be transferred from one location to another by glial as well as by neuronal mechanisms.

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Although the site of a large somatic storage pool of secretory material is a common characteristic of neurosecretory cells, it is not known if secretory proteins are withdrawn from this pool at random or in a specific transport and secretion, or if cellular mechanisms exist that route newly-synthesized proteins either to storage or transport. These alternatives can be tested by using neurons L11 and 815 of Aplysia, which synthesize and commit to axonal transport large quantites of a specific class of low molecular weight presumptive neurosecretory proteins (LMW proteins). Pulse-chase studies in which the LMW proteins are identified by SDS gel electrophoresis indicate that they leave the soma by a biphasic process: a rapid phase of decay (half-life 1-2hr) is followed by a slower phase (half-life >10hr). These kinetics are not due to the presence of more slowly turning-over contaminating species, since the same results are obtained when the LMW proteins are identified by migration on gel systems which separate proteins by both molecular charge and molecular weight. Two additional tests indicate that total LMW protein turns over at the slow rate: Exposure to vinblastine for 6hr blocks decay without affecting synthetic rate, but has no detectable effect on somatic LMW content as assayed by Coomassie staining of SDS gels. All LMW proteins were lost from the labeled higher molecular weight species is the same after 10hr of exposure to labeled precursor as after a 2hr exposure, whereas if total LMW protein is isolated at a 1-2hr half-life, the ratio should have declined significantly over this period. These data are not consistent with the single-pool model. Rather, they indicate that following synthesis these proteins are partitioned into at least two kinetically distinguishable somatic pools, one of which is rapidly subjected to axonal transport, and the other of which represents a slowly turning-over somatic storage pool.

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Slow Component b (SCb) is a complex constellation of proteins moving in the anterograde direction along the axon at a rate of 5–8 μm/day. Materials associated with SCb have been shown to move separately from the microtubule-neurofilament network (Slow Component a) and can include both axon and cell body (Carr and Laske 1978 Trans. Am. Soc. Neurochem. 9, 200. Black and Laske 1977 Soc. Neurosci. Abstr. 7, 299). Pulse labelled SCb is known to move for weeks or months as a discrete wave. These properties of SCb suggest that it represents a distinct cellular structure which may be termed the axoplasmic matrix, although some 60% of the proteins in SCb are easily solubilized in physiologic buffer. These easily solubilized proteins are commonly thought to be diffusible proteins in the cell and may include the enzymes of glycolysis and related intermediary metabolic pathways. Margarets et al. (1975 Biochem. Biophys. Res. Comm. 65, 369) reported the axonal transport of NSE and a second enzyme associated with energy metabolism, creatine phosphokinase (CPK) [EC, No. 2.7.3.2].

35S-Nethionine was injected into the eyes of adult guinea pigs. The animals were sacrificed 6 days later when the only labelled proteins in the optic nerve are associated with SCb. The optic nerve was homogenized and aliquots subjected to two dimensional electrophoresis. NSE was purified by a modification of the procedure of Margarets et al. (J. Biol. Chem. 241, 3645) and rabbit brain CPK obtained from Sigma (St. Louis, Mo.). Purified tubulin was subjected to polymerization/depolymerization and examined the binding of α-3H-BTX B to this protein. At a concentration of 0.1 μM, 3H-BTX B did not bind at the sodium channel site as BTX, this may not be the case with respect to toxin action on axoplasmic transport. The effect of β-tubatoxin benzate on axoplasmic transport should be tested in vitro.


Studies with HRP as a tracer have suggested that the agranular reticulum is a part of the anterograde axonal transport of peroxidase. We have reported (J. Comp. Neurol. 155:31, ’79) that in the hypothalamo-neurohypophyseal systems of the hypothalamo-neurohypophyseal system the uptake of HRP-containing acid hydrodase is more prevalent during anterograde axonal transport, but only in hyperosmotically stressed mice. We propose that these cisterns are part of the lysosomal system and that anterograde transport of HRP is associated with the movement of acid hydrodases, presumably from perikaryal secondary lysosomes. We report here additional evidence that these organelles in the soma participate in the anterograde movement.

In supraoptic perikarya from normal, hydrated mice injected with HRP into the blood or cerebral ventricles, very few agranular reticulum-like cisterns, attached or unattached to secondary lysosomes, contained peroxidase. The number of HRP-labeled cisterns and dense bodies increased when the animals were hyperosmotically stressed by having them drink 25% NaCl 5–6 days prior to HRP injection. Supraoptic cell bodies from omostructically stressed mice not injected with HRP were incubated for acid hydrodase activity using trimethylphosphate or β-glycerophosphate as substrate. Acid hydrodase–positive lysosomes and 400–1000Å wide cisterns proliferated in these perikarya. Some of the cisterns were called with HRP, and these were HRP-exposed parikarya incubated for both HRP and acid hydrodase activity from the salt-treated animals contained secondary lysosomes and a few cisterns which exhibited reaction products of both enzymes.

Our results suggest that agranular reticulum-like cisterns in the perikaryon, like those in the axon, are part of the lysosomal system of organelles and may provide the route through which acid hydrodases and other lysosomal-related substances can leave secondary lysosomes within the perikaryon for transport down the axon.


Neurons of the central visual pathways of the rat have been shown to take up horseradish peroxide (HRP) at the axon terminal and transport the protein retrogradely in a selective way (Bucht and Haschke, Neuron, 2, 1987). The rate of clearance of this material from the internal environment is of fundamental importance to a thorough understanding of the mechanism of neuronal transport. Our results suggest that agranular reticulum-like cisterns in the perikaryon, like those in the axon, are part of the lysosomal system of organelles and may provide the route through which acid hydrodases and other lysosomal-related substances can leave secondary lysosomes within the perikaryon for transport down the axon.
CALCIUM LOCALIZATION IN MAMMALIAN NERVE FIBERS IN RELATION TO ITS REGULATION AND AXOPLASMIC TRANSPORT. S.Y. Chan, S. Ochs, and R. J. Bild Jr. Dept. of Physiol., Anat., and The Biophysics Prog., Indiana University Medical Center, Indianapolis, IN 46223.

A specific Ca²⁺ requirement for axoplasmic transport was shown using a desheathed cat sciatic nerve (Ochs, S. Y. Chan, Nature, 270: 748, 1977). A block of transport was seen following incubation of nerves in a Ca²⁺-free or low Ca²⁺ medium with normal transport restored by adding Ca²⁺. The block in Ca²⁺-free media was considered due to a depletion of free Ca²⁺ from the axons. On the basis of the transport filament model, the level of free Ca²⁺ in the axon is regulated by mechanisms present in the mitochondria and endoplasmic reticulum (ER), which sequester and release Ca²⁺, keeping it close to about 10⁻⁷M. To show the presence of Ca²⁺, K⁺-pyroantimonate which binds to Ca²⁺ to form an electron-dense deposit, was used to stain thin sections of nerves for Ca²⁺. Nerves were incubated in media containing normal levels of Ca²⁺ or media with raised or lowered levels of Ca²⁺. Incubation was carried out at 38°C, pH 6.7 and the nerves bubbled with 95% O₂ + 5% CO₂. The nerve segments were fixed in 5% glutaraldehyde and 1% osmium with 2% K-pyroantimonate added. Preliminary studies using X-ray microanalysis also showed Ca²⁺ present in the granules. Experiments with Na⁺ and K⁺ showed very much less binding to pyroantimonate, when incubated in a Ca²⁺-free medium for 3 hours or more, the nerves were depleted of their granular deposits. Conversely, when desheathed nerves were placed in vitro media containing 20 mM Ca²⁺ or more, a larger number of granules was seen throughout the axoplasm, in the ER, inside the matrix and attached to the outside of the mitochondria. Granules were also seen in the nodules of Ranvier. This is related to the change in the level of Ca²⁺ at the nodal membrane. The increased deposits seen in the nerves exposed to high levels of Ca²⁺ were depleted from nerves returned to a low Ca²⁺ medium for at least ½ hr. This was most marked for the mitochondria and ER. Such changes indicate that the granules act to regulate the level of free Ca²⁺ in the axons by sequestering and releasing Ca²⁺.

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The microtrabecular matrix of axoplasm was found to consist of an organized system of crossbridges between microtubules, neurofilaments, cisternae of the smooth ER and the plasma membrane. It is proposed that formation and deformation of this system is involved in rapid axonal transport. In order to facilitate electron microscopic visualization of the trabecular connections between elements of axoplasm the following three techniques were used: First, the addition of tannic acid to the primary fixative (Ochs, in press). Second, embedding tissue in polyethylene glycol for thin sectioning, dissolving out the embedding media from the sections and then critical-point-drying (Wolosewick & Porter, in press). Third, visualization in freeze-etch replicas by rotary shadowing. All of these procedures yielded images of cross-linking elements between neurofilaments and organelles of the axoplasm.

Our next objective involved the determination of fixation conditions which best preserved the cross-linking trabeculae. We examined the effect of variable Ca²⁺ ion concentrations and low temperature upon the form and connectivity of these structures. By incubating nerve fibers in solutions containing either no Ca²⁺ with ionophore A23187 (Lilly) and EGTA, or alternatively high Ca²⁺ and ionophore, we were able to determine the effect of high and low Ca²⁺ ion concentrations upon the cross bridges. The results indicate that elevated reduced redox state and integrity of trabecular linkages between fibrous proteins and cisternal elements, while decreased Ca²⁺ availability within the axon increases the amount of intact axonal cross-links. These improvements in visualization should enable us to examine the distribution of trabecular links on motile axonal organelles.

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Transneuronal transport of some macromolecules such as radioactive tetanus toxin has been shown after retrograde intraaxonal transport (Schwab and Thoenen, 1976 Brain Res 105: 213-227). However, it has not been possible to detect transneuronal transfer of other materials such as labeled nerve growth factor although it is transported retrogradely in a similar manner (Schwab and Thoenen, 1977 Brain Res. 122:459-474). These data seem to favor the concept of a specific transneuronal transport of macromolecules. We now report a transneuronal transport of HRP in the ciliary ganglion of the rat. The HRP is known to be transported mainly in the retrograde direction.

Injections of HRP (1mg/μl, Sigma type VI) were made in the ciliary body of the eye. After 24 hours the animals were perfused and the HRP activity in the brain and in the ciliary ganglion was revealed by the Graham and Karmovsky technique (1966).

In the brains no HRP positive neurons were identified neither in the Edinger-Westphal nucleus nor in any other mesencephalic structure in the 40μ thick sections studied by light microscopy.

The electron microscopic observations on the ciliary ganglion homolateral to the injection showed HRP reaction product in the neuronal soma but the major proportion of HRP at this time interval was found at both dendritic processes and preganglionic axons. It was possible to visualize the release of the HRP reaction product from membrane-bound vesicles located in the peripheral perikarya or in the dendritic processes to the extracellular space. Also, in some cases images appeared in which vesicles containing HRP were incorporated by the preganglionic axon terminals. Such images were never observed in the synaptic cleft in our material.

Although the possibility of a trans-synaptic passage can not be ruled out, our results support the suggestion that the HRP is released from the ciliary ganglion cells to the extracellular space. This release seems not to occur at the synaptive surface. When the HRP is in the extracellular space, it is taken up by the axon terminals in the proximity but not in the region of the synaptic surface.

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This stimulation was found to depend on the level of Ca²⁺ concentration. Blockage of the stimulation of Ca²⁺-Mg²⁺-ATPase activity which was stimulated by incubation of the microsomes in 0.05% Triton X-100 was relatively small. The pellet obtained in this preparation contains an appreciable amount of brain microsomes. Cat brains were homogenized in 0.32 M sucrose and the post-mitochondrial supernatant fraction was incubated in 0.05 M Tris-HCl, pH 7.5 containing 10 mM CaCl₂, 1 mM MgCl₂, 200 mM sucrose, 100 μg/ml aprotinin, 20 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride for 30 min at 24°C. The microsomes obtained in the absence of Triton X-100 had Ca²⁺-Mg²⁺-ATPase activity which was stimulated by 1.5-5 fold by the calmodulin prepared from peripheral nerve. This stimulation was found to depend on the level of Ca²⁺ concentration. In the incubation medium, addition of TFP blocked the stimulation of Ca²⁺-Mg²⁺-ATPase. The ATPase activity responsible for flagellar beating, binds to brain microsomes and causes their ATP dependent cross-bridging. When, on the other hand, microsomes polymerized in the absence of dynein can, therefore, be used to determine the polarity of microtubules in situ.

We have used immunoperoxidase techniques to determine the localization of two axonally-transported polypeptides, designated 26 and 27, were previously shown to move down axons from dorsal column nuclei (DCN) at a velocity of approximately 50 μm per day, i.e. slower than the most rapidly moving group of axonally transported polypeptides (henceforward referred to as the 26-27 antigens) and twenty-seven (molecular weights 260,000 and 245,000, respectively) have an electrophoretic mobility similar to that of erythrocyte spectrin, smooth muscle filamin and several other actin-binding proteins.

In order to raise an antibody against 26 and 27, we purified these polypeptides from detergent extracts of guinea pig brain by means of gel filtration and preparative SDS gel electrophoresis and injected the denatured purified polypeptides into brain by means of gel filtration and preparative SDS gel electrophoresis and injected the denatured purified polypeptides into rabbits. The resulting antibody was specific for 26 and 27 by the criterion of immunodiffusion; in addition, it precipitated only 26 and 27 from detergent extracts of guinea pig brain and from extracts of unmyelinated nerve fibers and tract containing radioactively labeled transported proteins.

We used the antibody to locate 26 and 27 in frozen sections of nervous tissue by indirect immunofluorescence. In sections of guinea pig spinal cord and brainstem containing axons cut in cross-section, the major fluorescent profiles were ring-like, indicating that 26 and 27 are most concentrated near the axolemma. The fluorescent profiles probably cannot be entirely accounted for by staining of the myelin sheath since large motor nerve fibers display a more diffuse pattern of fluorescent staining. The major fluorescent profiles were present in the retinal ganglion cell layer, the inner nuclear layer, the inner plexiform layer, the outer plexiform layer, the inner nuclear layer and the ganglion cell layer.

The antibodies stained a subpopulation of intrafusal fibers, but did not stain the adjacent muscle fibers. A number of non-neural tissues were stained with the antibody, including the testis, adrenal gland and ovary. In all cases, the immunofluorescent staining was specific for 26 and 27 antigens since similar fluorescent profiles were absent when tissue sections were treated with pre-immune serum, pooled antisera from normal rabbits, or pooled antisera from rabbits injected with pre-immune serum.

The reason for the preferential uptake of 3H-pro by glial cells and oligodendrocytes was that these cells become heavily labeled in the brain when DCN was injected with 3H-pro. This labeling pattern is distinct from that of the 26-27 antigens since similar fluorescent profiles were absent when tissue sections were treated with pre-immune serum, pooled antisera from normal rabbits, or pooled antisera from rabbits injected with pre-immune serum.

The reason for the preferential uptake of 3H-leu by glial cells is that the neuronal uptake sites for leucine are different from those for proline. The results strongly suggest that the mechanism is one of an active transport, perhaps associated with the plasma membrane, and is not dependent on passive diffusion.


Many investigators have reported that effector neural connections of the injection site can be demonstrated with horseradish peroxidase (HRP) histochemistry. While the retrograde transport of HRP is universally ascribed to an active process within intact neurons, the mechanism responsible for its anterograde displacement remains controversial. For instance, Zaborowski et al. (1974) consider the anterograde transport of HRP to be insignificant and unreliable while others stress the necessity for axonal damage and subsequent passive diffusion. However, we would like to present evidence which suggests that the anterograde transport of HRP is an active and reliable process, perhaps identical to the transport of this enzyme in the retrograde direction.

Experiments with intracocularly injected HRP in rats show that the anterograde displacement of the enzyme to the suprachiasmatic nucleus, to the lateral geniculate body and to the superior colliculus is blocked if the eye is pretreated with colchicine or pentobarbital. No blocking occurs when the pretreatment consists of saline. Furthermore, if the pretreatment precedes the HRP injection by 10 days, the block is no longer evident. Thus, the inhibition of anterograde displacement is reversible and not due to permanent injury of the retina. These results strongly suggest that the mechanism is one of active transport, perhaps dependent on the microtubular system.

In further experiments, the brains of rats with intraocular HRP injections were processed not only for HRP histochemistry but also with the Nauta stain. The absence of degeneration-induced axon pathfind to the same areas that received anterogradely transported HRP indicated that retrograde transport is not necessary for the occurrence of anterograde transport. In addition, neither the rapid rate of the appearance of the reaction product at anterograde termination sites nor its granularity is consistent with passive diffusion as the underlying process.

The validity of the anterograde transport of HRP can be shown by injecting HRP into the supranuclear portion of the suprachiasmatic nucleus of the hypothalamus and visualizing the efferent neural connections. This conclusion is supported by the fact that putative neurotransmitters in DCN, such as glycine, glutamate and GABA, all produced labeled microvascular profiles, and the retrograde transport of HRP into the DCN is a significant observation since it indicates that the active anterograde transport of HRP is an active process.

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COMPARISON OF PROTEINS TRANSPORTED IN DIFFERENT CNS TRACTS.
A comparison was made of the [35S]-methionine labeled proteins axonally transported in each of three central nervous system tracts in the rat, i.e., 1, retina to superior colliculus, 2, dorsal lateral geniculate body (dLg) to visual cortex, and 3, substantia nigra to "striatum" (nuc. caudatus, putamen, and nuc. accumbens). A portion of the retina, dLg or substantia nigra was killed by sacrifice at time points designed to coincide with the arrival of the fast (3-4 hr), intermediate (16-48 hr) and slow (6-21 days) waves in the respective projection sites. After dissection of the injection and projection sites and solubilization of the tissue, proteins were fractionated on a discontinuous (3% stacking gel) 7.0-19% polyacrylamide gradient slab gel in buffers containing sodium dodecyl sulfate. The [35S]-methionine labeled proteins were visualized by fluorography of the dried slab gels.

The injection site radioactive protein profiles were similar for the dLg and substantia nigra, and differed from retinal proteins labeled by intraretinal injection, especially in the intensity of labeling in the range of 35,000-45,000 daltons. During a time span from 3-4 hrs. to 6-21 days after isotope injection, the labeling pattern of the individual injection sites changed very slightly.

In contrast, time-dependent changes were observed in the projection sites; each transport wave contains characteristic proteins. In all three tracts the labeled proteins transported in each wave are very similar in electrophoretic pattern quantitatively. The only distinctive differences observed were labeled protein of Mr=27,000 (arriving in the intermediate and slow waves) absent in the superior colliculus but present in the visual cortex and absent in the substantia nigra; and a protein of Mr=15,000 (arriving in the slow wave) absent in the visual cortex but present in the striatum.

These heavily labeled rapidly transported proteins common to the three tracts exhibit similar turnover properties: one, Mr=105,000, has disappeared by the arrival of the intermediate wave and the other, Mr=26,000, has decreased considerably by the time of arrival of the slow phase.

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AFFERENT PROJECTIONS TO THE MEDULLA OBLONGATA IN THE CAT.
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The rostral to midcaudal extent of the medulla oblongata is known to mediate the integration of basic physiological processes including inhibitory motor effects and the coordination of the visual motor system. A recent report (Neurosci. Abst., Vol. 11 (111), 1977) described projections to the rostral gigantocellular tegmental field (FTG) using injections of 0.2 ul horseradish peroxidase (HRP) and incubating with diaminobenzidine (DAB) procedures.

We have been using the more sensitive tetramethylbenzidine (TMB) chromagen for incubations after preincubation with 25mM NaPT. Two heavily labeled rapidly transported proteins common to the three tracts exhibit similar turnover properties: one, Mr=105,000, has disappeared by the arrival of the intermediate wave and the other, Mr=26,000, has decreased considerably by the time of arrival of the slow phase.

Effects of changes in the composition of external solutions on structure and axonal particle transport in amphibian axons.

Deaerated sciatic nerves from Xenopus laevis were incubated for varying periods of time in salines with and without divalent cations. Single axons were sampled from the nerve bundles and these were viewed by darkfield microscopy to detect axonally transported particles. Nerve bundles treated similarly, but not incubated, were found to be normal.

Nerves incubated in salines containing (mM): NaCl, 112; KCl, 3.0; CaCl2, 2.0; MgSO4, 2.0, and buffer, displayed an active transport wave of rapidly transported particles. Nerve bundles treated similarly, but without divalent cations, were observed to have a normal structure for at least 12 hr. In solutions with no Ca2+ or Mg2+ and with 2 mM EDTA the axons showed, over a period of 4h, a slowing or arrest of particle transport concomitant with gross structural changes. The structural changes consisted of collapse of the axolemma and a beading of some of the fibers. Ultrastructural examination showed that in these fibers the axolemma contained defects which may represent holes or local deformations. Additional to 2 mM NaPT to the solution protected the axons from the structural changes and particle transport continued normally.

Nerves in isotonic NaCl or Na gluconate with EDTA showed slight collapse of the axolemma by 4h with continued particle transport. In isotonic KCl with EDTA the fibers became beaded by 4h; the axolemma and particle transport was active. Nerves in isotonic K gluconate with EDTA remained normal in structure and retrograde particle transport was observed at least up to 24h.

It is concluded that the abnormality of particle transport in nerves bathed in conventional physiological salines from which divalent cations are omitted is secondary to the structural changes in the axons. Solutions free of divalent cations which caused no or minimal structural changes in the axons caused no abnormalities of particle transport. Therefore, it is concluded that divalent cations play no axon-specific role in the transport of particle.
ON THE IMPORTANCE OF PROTEIN SYNTHESIS FOR AXONAL TRANSPORT


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These experimental results provide evidence that protein synthesis is necessary for the loading not only of amino acids but also of nonprotein precursors onto the transport system. Dorsal root ganglia of R. catesbeiana were incubated in (32P)-phosphate, (3H)-leucine, or (3H)-serine, and transport was allowed to proceed in the attached sciatic nerve for up to 20 hours. Liquid scintillation analysis revealed that the transport of all three isotopes was blocked when the ganglia were exposed to cycloheximide or puromycin before the isotope was added. If cycloheximide or puromycin were added after the isotope, the amount of activity transported increased as the delay between the addition of isotope and of inhibitor was increased. In the case of (32P), label was found in the TCA soluble and chloroform-methanol fractions.

The multiwire proportional chamber was used to follow the movements of (32P) and (35S). When either inhibitor was added several hours after either isotope, the results were similar to those obtained when the nerve was transected close to the ganglia a while after adding the isotope. Transport velocity was unaffected, but the export of material from the ganglia ceased abruptly following the addition of the inhibitor. These results are consistent with the notion that the loading of materials onto the transport system involves the formation of a lipid-protein complex, possibly in the form of membrane.

(Supported by the MRC of Canada)

IMMUNOCYTOCHEMICAL LOCALIZATION OF PLASMA PROTEINS IN NEURONAL PERIKARYA.


It is well known that exogenous protein tracers are transported intraxonally to neuronal perikarya following their uptake by axonal terminals. Foreign proteins shown to be included in transport are haemoglobin, albumin and tetanus toxoid, and nerve growth factor. This led us to speculate that there may be movement of naturally occurring plasma proteins in the axonal transport system in the blood-brain barrier. We hoped to find a valuable tool for studying peroxidase-anti-peroxidase (PAP) immunocytochemical technique.

The multiultrasonic rays were rapidly frozen in isopentane and liquid nitrogen. Sections (10 μm) were immersed in (35S)-Met, or (3H)-Leu, and transport was allowed to proceed for 10 minutes in 95% ethanol at room temperature. The following steps were then carried out at room temperature. (1) incubation of sections for 30 min. in rabbit anti-serum to whole rat serum at various dilutions in Tris-buffered saline (TBS) (pH 7.6). (2) washing as above. (3) application of goat antibody to rabbit IgG diluted 1:10 with TBS for 30 min. (4) washing as above. (5) treatment with rabbit PAP complex diluted 1:50 with TBS. (6) washing as above. (7) incubation in hydrogen peroxide and 33'-diaminobenzidine in Tris-buffer (pH 6.4) for 15 min. (8) finally sections were washed, dehydrated, cleared and mounted.

Reaction product, indicative of the presence of serum protein, was seen in neurons of hypoglossal nucleus, nucleus ambiguus and dorsal motor nucleus of vagus. A diffuse staining was also present in the nucleus of the area postrema, a region where the blood-brain barrier to protein is known to be absent.

Appropriate control sections were unstained confirming that staining by the complement reaction demonstrated the site of attachment of primary antisera to antigenic proteins normally present in the serum of rats.

These results suggest that one or more of the proteins of serum is normally present in central neurons whose axons terminate outside the CNS.


FAST AXONAL PLASMA TRANSPORT OF TUBULIN AND TRIAD PROTEINS

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Two axonal transport mechanisms are usually considered present in nerve fibers, one giving rise to a fast, the other to a slow downflow. Alternatively, in the "unitary" view, one transport mechanism accounts for both the fast and slow rates with the slow rate resulting from those materials dissociating from the transport mechanism early on (Ochs, J. Physiol. 255:459, 1975). The L5 dorsal root ganglia and ventral cord of rats were injected with high specific activity (35S)-methionine as a protein precursor, and the axoplasmic transport of labeled proteins in their sciatic nerves studied. The nerves, taken after periods of downflow of 4 hours to 33 days, were cut into 5 mm segments, each segment delipidated in chloroform: methanol (2:1) and solubilized in 8 M urea, 5% 2-mercaptoethanol, 1% SDS in 60 mM Tris- HCl, pH 6.8. The solubilized proteins of successive nerve segments were each electrophoresed on SDS polyacrylamide gels, stained, scanned and prepared for fluorautoradiography. The slow transported proteins contained the prominently labeled "triad" neurofilament proteins and a and β tubulin subunits (Hoffman and Laskey, J. Cell Biol. 66:351, 1975). Additionally, a smaller amount of tubulin and "triad" proteins were found to be fast transported in the crest, at close to 401 mm/day. The fast transported tubulin and "triad" proteins amounted to about 5% of the slow downflow. The tubulin and "triad" proteins in the crest were higher in amount than in the plateau region just behind the crest. The difference in the specific activity of tubulin in the crest and the plateau region was more pronounced when a postganglionic ligature was placed 2 hrs. after the injection of [3H]-leucine into L7 dorsal root ganglia of cats and sciatic nerves taken after 5-7 hrs. of downflow. The nerve portions corresponding to the crest and the plateau regions were prepared for analysis of tubulin by the temperature dependent assembly-disassembly procedure and the amount of tubulin in TCA insoluble. These procedures also demonstrated that a small amount of tubulin and "triad" proteins are transported in the slow transport system, and that these proteins may be diluted by the increase in the amount of tubulin and "triad" proteins in the slow transport system.

Glia-Axon Protein Transfer: A SELECTIVE PROCESS WHICH SUPPLEMENTS THE SUPPLY FROM THE NEURON SOMA.

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In the squid giant axon, there is considerable evidence that some of the proteins synthesized by the adaxonal glial cells are actively transferred into the axon, but little is known about these putative transferred proteins. In order to obtain a better understanding of their properties and functions, we compared them with the major proteins of axoplasm (AXM) and the proteins synthesized by the glial sheath and the satellite ganglion. Axoplasm proteins were prepared by dissecting a segment of the sciatic nerve, which consists of the soma. Proteins synthesized by the sheath and ganglion were labeled by incubating 3-4 cm of ligated giant axon and the isolated ganglion in 0.3 ml of artificial seawater with 0.5 μCi of three labeled amino acids: 3H-leucine, 3H-lysine and 3H-ornithine for 60-770 min at 19-21°C. At the end of the incubation, the axon and ganglion were rinsed and the AXM, containing labeled transferred proteins, was extruded. The empty sheath, AXM, and ganglion were then homogenized in solubilization buffer (5% β-mercaptoethanol, 8 M urea, and 10 mM Tris-HCl, pH 7.3). All homogenates were centrifuged at 100,000 g for 30 min to remove insoluble material and frozen until analyzed by combined iso-electric focusing and SDS-polyacrylamide gel electrophoresis (2D-PAGE).

The 2D-PAGE pattern of AXM revealed more than 50 labeled proteins and was similar to the sheath pattern. However, one of the most highly labeled sheath proteins was not transferred at all, whereas other minor labeled species of the sheath appeared as prominent labeled proteins in the AXM. Thus, the transfer process is selective.

About 19 proteins in the 2D-PAGE pattern of AXM were stained with Coomassie blue, indicating that they are major axonal proteins. Eleven of these proteins were stained the same as the axonal labeled transferred proteins, which suggests that some of the transferred proteins are identical to major axonal proteins. Because the ganglion is the source of some of these major axonal proteins, we then compared the proteins synthesized in the ganglion with the transferred proteins and found that several of the transferred proteins, which were identical to axonal proteins, were present in the ganglion. These observations show that some of the major proteins of axoplasm, presumably supplied principally by the soma, are also selectively transferred to the axon by a fast rate.

Therefore, we propose that glia-axon protein transfer may serve to supplement a specific fraction of proteins supplied to the axon via axonal transport from the soma.

Horseradish peroxidase (HRP, Sigma Type IV, 25%) was applied microiontophoretically to the lateral preoptic area (LPA) of male hooded rats. After 24 hr animals were perfused intracardially, the brain was removed and sectioned in the frontal plane. Sections were processed with DAB for the brown reaction and were lightly counterstained with cresyl violet. Labeled neurons were identified and photographed under the microscope in light and dark fields and sequential sections were examined. The HRP ejection sites were approximately 500 μm in diameter within the LPA. Anterior to the ejection site a large number of labeled neurons and axons were located in the LPA and medial forebrain bundle (MFB). Many labeled axons and cell bodies were found along the stria hypothalamic tract dorsal to the ejection site. Labeled soma were found in the globus pallidus and ventral caudate putamen. In addition, labeled axons and soma were observed along the stria terminalis and diagonal tract of Broca into the lateral septal nucleus. Axons were also labeled in the anterior commissure. Labeled neurons were located ventral and lateral to the ejection site in the olfactory terberculum. Also, cell bodies were labeled in the medial preoptic nucleus. The only organelles which appeared to be specifically adsorbed to anti-H coated immune-affinity grids were filaments of approximately 100 Å diameter; in some cases the anti-H IgG adsorbed more than 100 times as many of these filaments as did control grids which had been incubated with the same concentration of IgG from a non-immunized goat. The capacity of the anti-H IgG to adsorb filaments onto grids was completely blocked when the IgG was preincubated with H.

These experiments provide immunological evidence that H, a polypeptide which is axonally transported in the most slowly-moving transport group (group V) of the rabbit retinal ganglion cells, is associated with the 100 Å filaments of neurons. H is a 195,000 molecular weight polypeptide which, together with at least two other polypeptides (45 and 46, molecular weights = 145,000 and 73,000, respectively) is axonally transported at a velocity of 0.7-1.1 mm per day in the rabbit retinal ganglion cells. At least four other groups of proteins are transported at more rapid velocities in these neurons.

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These experiments provide immunological evidence that H, a polypeptide which is axonally transported in the most slowly-moving transport group (group V) of the rabbit retinal ganglion cells, is associated with the 100 Å neurofilaments. Group V therefore may be largely concerned with the movement of neurofilament-associated proteins, as was originally proposed by Hoffman and Lasek for a similar transport group (SCa) in rat sciatic nerve. Our experiments also serve to demonstrate the potential usefulness of immune-affinity electron microscope grids as a general approach for determining the organelle association of certain proteins.
BASAL GANGLIA
Investigation of rostral vestibular projections has led to the hypothesis that the caudate nucleus may mediate egocentric spatial orientation behavior through proprioceptive afference (Potegal, Acta Neurobiol. Exp. 32: 479, 1972). This hypothesis was tested with a spatial task allowing only such afference (Potegal, Acta Neurobiol. Exp. 32: 479, 1972). This axon of a large aspiny neuron (soma 67 μm in diameter) coursed caudally. It could not be followed beyond the border of visual, auditory, tactile, and olfactory directional cues. The axon employed vestibular afference in the performance of righting reflexes (as a measure of vestibularly-based spatial orientation behavior). These findings support the hypothesis that the caudate nucleus employs vestibular afference for egocentric spatial orientation to the environment, while the caudate mediates the use of proprioceptive cues for egocentric spatial orientation.

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In our previous study (Preston et al., 1979) the axons of several neurons originating in the neostriatum of rats to the globus pallidus (GP) or internal segment of the globus pallidus (SN) were followed. The axons of at least three different neostriatal neurons have been described previously (Preston et al., 1979). One group received bilateral radiofrequency lesions (0.1-mm diameter); the third group received no lesion and served as a sham operation control group. Lesions in one group were placed in the posteroventral portion of the caudate nucleus; a second group were placed in the hippocampus, an area implicated in some spatial tasks but which has not been reported to receive direct vestibular or other proprioceptive afferences. Following a period of post-operative recovery, all animals were retested for eight days. Comparison of pre-operative to post-operative scores revealed improved performance above and beyond the sham and the hippocampal groups. The caudate group, however, had significant performance deficits. These deficits were evident in maximum path length achievement and in mean number of errors.

These results indicate that in terms of two parameters, fine structure and Ach levels, the thin slices are closer to the in vivo state.

The refinement of the brain tissue slice technique for neurophysiology has made it possible to measure electrical activity from the thin slices in response to such factors as injections of neurotransmitter agents. Previous autoradiographic 14C deoxyglucose (DG) studies (Brown and Wolfson, 1978) found that apomorphine increased glucose utilization in several extrapyramidal system nuclei: the substantia nigra reticulata (SNR), subthalamic nucleus (STN), globus pallidus and striatum. The substantia nigra complex (SNC) was apparently unaffected. The localized increase in glucose utilization (GU) in several extrapyramidal system nuclei and subnuclei differently. Supported by NIH grant NS 09649.

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In the present study, the same technique has been used to examine specific details of the axons of several neurons originating in the neostriatum. For the purpose of autoradiography, the brains were prepared for autoradiography following the methods of Sokoloff et al. (1977). Seven animals were anesthetized with halothane but not treated with apomorphine. Autoreradioactivity and electron microscopic studies of apomorphine effects in halothane anesthetized rats. Lucy L. Brown, Leslie L. Wolfson, Dept. Neurobl., Albert Einstein Coll. of Med., Bronx, N.Y., 10461.

Previous autoradiographic 14C deoxyglucose (DG) studies (Brown and Wolfson, 1978) found that apomorphine increased glucose utilization in several extrapyramidal system nuclei: the substantia nigra reticulata (SNR), subthalamic nucleus (STN), globus pallidus and striatum. The substantia nigra compacta (SNC) was apparently unaffected. The localized increase in glucose utilization (GU) in several extrapyramidal system nuclei and subnuclei differently. Supported by NIH grant NS 09649.
EFFECTS OF NEONATAL MEDIAL FOREBRAIN BUNDLE (MFB) LESIONS ON DEVELOPMENT IN CATS. II. PREVENTION OF AMPHETAMINE-INDUCED SLOWING OF THE SPONTANEOUS FIRING RATES OF CAUDATE NEURONS BY NEONATAL MFB LESIONS. W.A. Levine, M.T. Levin, J. H. Carter, R. M. Brown, and E. Garcia-Rill. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA. 90024.

We have recently described some of the behavioral, neurochemical and electrophysiological effects of MFB lesions made in kittens of 9-21 days of age (Levine et al., Neurosci. Abst. 3 (1977) Levine et al., Neurosci. Abst. 4 (1978)). While the present experiment assessed the neurophysiological effects of amphetamine treatment on the spontaneous firing rates of caudate neurons and determined if these effects could be blocked by neonatal MFB lesions that interrupt the nigrostriatal pathway and severely deplete the caudate nucleus of dopamine. Juvenile cats of 7-12 months of age received 3 doses of amphetamine sulfate (1.24 mg/kg i.p.). Time between injections varied from 10 days to several weeks. The spontaneous firing rates of caudate neurons were measured several weeks to several months after the last amphetamine injection. The cats were then sacrificed and the dopamine content of the caudate nuclei determined. In intact cats amphetamine produced a slowing in the spontaneous firing of caudate neurons. Average interpulse interval (ISI) in amphetamine-treated cats was about 3800 msec compared to about 1800 msec in intact cats. In cats that had received neonatal MFB lesions testing for the dopamine content by about 75%, amphetamine treatment did not slow the firing rates of caudate neurons. Average ISI for amphetamine-treated cats with MFB lesions was about 2200 msec. This result was not a nonspecific effect of neonatal brain damage since amphetamine produced a decrease of about 15% in the spontaneous firing rates of caudate neurons in a group of cats that had ventral thalamic lesions as neonates (Mean ISI=3900 msec). In addition to its effects on the spontaneous firing rate, amphetamine treatment produced a 40% depletion of caudate dopamine in unlesioned cats. These results indicate that in the developing cat, a relatively mild form of dopamine deficiency produces long-lasting neurophysiological and neurochemical effects. Furthermore, the neurophysiological effects can be blocked by neonatal MFB lesions that interrupt the nigrostriatal pathway.

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Investigations using the 14C-deoxy-d-glucose technique have found that the dopamine agonist apomorphine and d-amphetamine produce many effects in rat striatum of metabolic activity in the subthalamic nucleus (STN) (Woolson and Brown, Soc. for Neurosci. Abst. 2:510, 1976 and Wecker, et al., J. Neurochem. 32:15, 1979). In addition, iontophoretic administration of dopamine to STN neurons produced excitation of dopamine-sensitive neurones. The current study was undertaken to identify afferent connections to the STN using the horseradish peroxidase (HRP) technique.

Male Sprague-Dawley rats (140-160 gms.) were anesthetized with sodium pentobarbital (60 mg./kg.), placed in a stereotaxic frame, and surgically prepared by removal of skull, dura, and cortex over the electrode site. In some experiments, single-barrel micropipettes were used, while in others four-barrel micropipettes were used to permit passage of a negative holding current through the HRP barrel during injection and withdrawal. Electrodes were inserted stereotaxically using the atlas of König and Klippel. Four percent HRP (Sigma Type VI) in pH 8.5 Ringer-Lyta buffer was injected using pulsed positive currents (1 sec. pulses, freq. 0.5 Hz, amplitudes up to 1000 ul for time periods of 65 to 90 min.). After survival periods of 24 to 30 days, the brain was processed histologically using the tetramethylethylenediamine technique of Mesulam (J. Histochem. and Cytochem, 26:106, 1978).

Labelled axons were observed extending from STN to the ipsilateral cerebral peduncle running along a rostral-caudal axis, and from STN toward substantia nigra. Labelled cell bodies were observed in the lateral SN, zona incerta, and globus pallidus. In addition, a large percentage of cell bodies were labelled in the pars compacta region of the ipsilateral substantia nigra. Label was observed in only a few cells of the pars reticulata. No labelled cells were observed bilaterally. These observations suggest the existence of a pathway from the pars compacta region of the substantia nigra to STN. The data also further document the known pathway from the globus pallidus to the STN. These findings provide anatomical evidence consistent with a dopaminergic input to STN.

THE BASAL GANGLIA-TECTAL PATHWAY I: ITS ROLE IN VISUALLY GUIDED BEHAVIOR IN THE PIGEON. Nellie M. Ruben* and William Bodis. (Spon: Roger M. Brown) Department of Psychology, University of Nebraska-Lincoln College of Arts. 11. E. Grinnell St., Lincoln, Nebraska 68588.

In birds, reptiles and mammals, neural output of the basal ganglia is relayed to the tectum. In birds, this prominent pathway originates in the paleostriatum primitivum (the avian equivalent of the globus pallidus) and synapses in a large diencephalic nucleus, spiriformis lateralis (SpL). SpL then projects to widespread regions of the optic tectum (Karten & Hubelh, J. Comp. Neurol., 148, 1973; Brecha, Hunt & Karten, Neurosci. Abstr., 1976).

The present study investigated the function of this pathway in pigeons. Pre-operatively, subjects were trained to: (1) discriminate visual stimuli differing in color, intensity or pattern; 2) track a target which changed position continuously (i.e., neck grain mounted on a revolving drum); 3) track a target which changed position intermittently (i.e., rapidly peck a sequence of response keys which were illuminated in a random order); and 4) peck stationary targets (grain mounted in the same position as the response keys). Subjects were also examined on a series of neurological tests and assessments of normal locomotion patterns. Following training, the basal ganglia-tectal pathway was interrupted in 10 subjects by placing electrolytic lesions in SpL; in 8 other subjects, control lesions were placed in nucleus rotundus, nucleus ovoidalis, or nucleus pretectalis. Subjects with bilateral interruption of the basal ganglia-tectal pathway showed a severe and seemingly permanent deficit in their ability to track targets which changed position in space. In the task in which the target shifted position intermittently, subjects continued to peck the response keys, but took much longer to complete a response than normal control subjects. In the task in which the target moved continuously, most subjects were totally incapable of locating the grain when it moved at the lowest velocity (8cm/sec). These same subjects were able to detect and accurately peck stationary targets, and to discriminate visual patterns, intensity and color. Motor behavior, including flight, walking, and head movements were normal in these subjects, as were neurological reflexes related to posture and locomotion. Control subjects were not impaired on any tasks.

These results indicate that in normal birds, the basal ganglia provide information to the tectal critical to the ability to respond to changes of the spatial position of visual targets. This pathway may relay information about the bird's ongoing or impending movement to the tectum so that prior to the initiation of a peck, sensory input concerning the spatial location of a target can be correlated with the subject's own body position.

CELLS OF ORIGIN OF DIFFERENT PALLIAL DIFFERENTIATES ARE SEPARATE. David A. Carter* and Derek van der Kooy (Spons: F. Coenen) Toronto General Hospital and Department of Anatomy, University of Toronto, Toronto, Canada.

Experiments in the cat have demonstrated entopeduncular nucleus (EP) cells are more likely to project to either ventralateral—ventral anterior nuclei of thalamus (VAL), centromedian—parafascicular nucleus (CM-PF), lateral habenula (LHB) or nucleus tegumentoideum irrespective of any topographical specificity of these neurons within EP. Carter and Fibiger (1976) suggested that the EP projection to TIP is neither ventral nor medial in location since the injection of HRP in the EP terminal region did not project the same results when the injection into the thalamus. In order to re-examine this problem the fluorescent retrograde double labeling technique was developed by van der Kooy, Koper, and Catanas-Ferrevoet (1976) has been utilized. Fifteen rats had 0.1-0.3 ul HRP—Primuline injected into one EP terminal field and the same amount of Evans Blue injected into another EP terminal field. The EP terminal field included VAL, LHB, and the TIP region. Injections into LHB labeled virtually all EP neurons within the rostral two-thirds of this nucleus, but resulted in minimal labeling in its caudal aspects. This caudal region, however, contained a circumcresed group of cells labeled after injections into VAL. The TIP region injections resulted in heavy labeling in zona incerta, lateral hypothalamus, and a band of cells which runs ventral to EP to mesothalamus into the central nucleus of the amygdala. These findings suggest that different pallial outflows represent functionally and anatomically distinct basal ganglia processes.

Combining the techniques of intracellular recording and intracellular labeling with horseradish peroxidase (HRP) with light and transmission electron microscopy (TEM), we have identified several neuronal types in the rat neostriatum. Intracellular records were obtained from these neurons following stimulation of the cell bodies of the neostriatum (CX) and the substantia nigra (SN). Recording glass microelectrodes were filled with 4% HRP in 0.5 M KCl-Tris buffer (pH 7.6). HRP was injected by passing positive DC pulses (3-5 nA, 100-400 msec duration, frequency 2-5/sec) through the recording electrodes. After fixation and histochemical processing, the HRP labeled cells were analyzed under the light microscope and subsequently processed for TEM.

The most frequently encountered cell type was a medium spiny neuron which responded with monosynaptic excitatory postsynaptic potentials (EPSPs) to stimulation of CX and SN. The other cell types included large and medium aspiny neurons, the former responded with EPSPs to SN stimulation and the latter with monosynaptic EPSPs to CX and SN stimulation. Under TEM, these neurons displayed differences with respect to their nuclear morphology (nucleus size, nuclear invaginations, and heterochromatin distribution), and the shape, size and distribution of their rough endoplasmic reticulum (ER), smooth ER, ribosomes, Golgi apparatus, lysosomes and mitochondria. For instance, the nucleus of the medium spiny neuron was round with no membrane inclusions while those of the large and medium aspiny cells had many dense membrane invaginations. These latter two aspiny neurons could be distinguished from each other on the basis of differences in the nuclear-cytoplasmic ratio and the overall soma size.

In conclusion, we have positively identified the morphological characteristics, at both the light and ultrastructural levels, of at least three different neuronal types in the rat neostriatum and recorded differences in their intracellular responses to extrinsic inputs. (Supported by USPHS Grant NS 14866 and in part by NIH NRRG RR 0572-04).

THE POSTNATAL DEVELOPMENT OF PRECRUCIATE CORICOSTRIATE PROJECTIONS IN KITTENS. J.A. Cospito, M.S. Levine and A.M. Aminoff. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, Ca. 90024.

The purpose of this study was to investigate the organization of corticostriate connectivity in kittens at early postnatal ages. We reported previously that projections from the medial and lateral precruciate cortex in young animals are prominent and differentially organized within the lateral half of the head of the caudate nucleus and dorsal aspects of the rostral putamen. Results from this study were based on Fink-Heimer staining of preterminal and terminal degeneration following selective destruction of precruciate cortex. It was difficult to identify terminal degeneration with this argyrophilic method in young brains. Therefore, we decided to repeat the study by using the autoradiographic technique. Multiple injections of a mixture of tritiated leucine and amino acid precursors were made into the precruciate gyrus of twelve kittens, ranging in age from two to twenty-seven days. As with the Fink-Heimer technique, the precruciate projections to the head of the caudate nucleus were prominent and restricted to the lateral half. However, using autoradiographic labeling techniques, we observed that 1) the projections to the head of the caudate nucleus display a patchy distribution, 2) cortical projections extend caudally into the body of the caudate nucleus and remain patchy in character, 3) the projections to the putamen appear to be more prominent than observed with the Fink-Heimer method, and 4) contralateral corticostriate projections are organized similarly to ipsilateral projections. From the striatal areas with the greatest cortical terminal fields, cores of tissue were removed we observe that 1) the projections to the head of the caudate nucleus and SN were prominent and restricted to the lateral half. From the striatal areas with the greatest cortical terminal fields, cores of tissue were removed and recorded differences in their intracellular responses to extrinsic inputs.

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The pathology of Huntington's disease (HD) involves the degeneration of striatonigral GABA neurons and the loss of intrastriatal cholinergic neurons. It has been demonstrated that the intrastria-tal administration of the excitotoxin kainic acid (KA) produces a lesion which closely mimics the biochemical changes in HD. Although this animal paradigm provides insight into the neuropathological and biochemical changes in HD, it is not clear whether this model may be pharmacologically analogous to the human counter part. We now report studies on the pharmacology of the KA animal model. Subjects were Shaker-1 mutant mice, male Sprague-Dawley rats stereotactically placed injections of KA into the caudate-putamen nucleus. Animals were killed for stereotyped behavior using a five point rating scale. Injections into the substantia nigra produced both behavioral stimulation of d-amphetamine (2.0 mg/kg), in a dose per se subthreshold. As with the Fink-Heimer technique, the projections to the head of the caudate nucleus were prominent and restricted to the lateral half. However, using autoradiographic labeling techniques, we observed that 1) the projections to the head of the caudate nucleus display a patchy distribution, 2) cortical projections extend caudally into the body of the caudate nucleus and remain patchy in character, 3) the projections to the putamen appear to be more prominent than observed with the Fink-Heimer method, and 4) contralateral corticostriate projections are organized similarly to ipsilateral projections. From the striatal areas with the greatest cortical terminal fields, cores of tissue were removed and recorded differences in their intracellular responses to extrinsic inputs.

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In conclusion, we have positively identified the morphological characteristics, at both the light and ultrastructural levels, of at least three different neuronal types in the rat neostriatum and recorded differences in their intracellular responses to extrinsic inputs. (Supported by USPHS Grant NS 14866 and in part by NIH NRRG RR 0572-04).
ON THE ROLE OF GLOBUS PALLIDUS AS A SOURCE OF GABA-ERGIC PROJECTIONS TO THE NIGRA. G. Di Chiara*, M. Morelli*, M.L. Porceddu and M. Del Fiacco* (SPON-W.RCollu) Institute of Pharmacology, University of Cagliari, Italy. It is well known that long GABA-ergic neurons project from basal ganglia to the substantia nigra. While it is generally agreed that this pathway originates, at least in part, from the caudate-putamen, there has been much debate on the role of the globus pallidus. Although this role has been reaffirmed by various recent studies, the evidence provided until now is either indirect or derives from non-specific pallidal lesions. In order to reexamine this problem we studied in rats the effect on nigral GAD of pallidal and striatal lesions induced by local injections of kainic acid, which destroys neuronal perikarya but spares axons "en passage". Unilateral intrapallidal injection of kainic acid (0.75 μg/5 μg) resulted in loss of neuronal perikarya in most of the pallidus, in the MFB area, in the anterior thalamus adjacent to the internal capsule (reticular nucleus) and in the most ventral part of the caudate body. The head and the dorsal 2/3 of the body of the caudate were intact. This lesion resulted in a non-significative decrease (-8%) of nigral GAD on the lesioned side. Kainic-lesions of the dorsal caudate body instead, resulted in a dramatic (-52%) reduction of nigral GAD. Kainate-lesion of the caudate head resulted in a significative (-18%) reduction of nigral GAD. Kainate-lesions of the caudate tail failed to reduce significatively nigral GAD.

These data negate the existence of a GABA-ergic pallido-nigral projection but confirm that the caudate head contributes only a minor component to nigral GABA-ergic afferents as compared to the dorsal caudate body.


The neuropil of the medial and lateral segments of the globus pallidus was examined in serial sections. Results show that the densities of uniformly impregnated nigral neurons contain a larger number of small, round, non-dense small clear vesicles. Some of these vesicles, are surrounded by synaptic axon terminals and both are encapsulated by glial processes. Some dendrites are not surrounded by axons and only occasionally are postsynaptic.

At least four types of profiles containing vesicles are observed. (1) The most numerous are small boutons, about 1 μm containing large clear and dense core vesicles and forming symmetric contacts. They project fine finger-like processes, frequently devoid of vesicles, deeply inside adjacent elements of similar type. Contiguous elements of this category may show symmetric membrane densities with a widened intermembrane gap. These terminals arise from fine collaterals of small caliber myelinated axons (0.5 μm) and most likely correspond to the fibers of striatal origin seen in Golgi material. (2) Large bulbous elements, up to 3 x 4 μm, contain loosely packed small pleomorphic vesicles, cisterns and numerous mitochondria. Each bout contains multiple dense core vesicles with a single large electron dense vesicle or one contact with each of several smaller profiles. They are seen to emerge from large caliber myelinated axons (1-1.5 μm) which course in bundles with gold boutons. Such fibers may correspond to the second category of afferents seen in our Golgi material. (3) Small to medium size profiles (1-2.5 μm), have many small round vesicles and numerous mitochondria. Some contain thin myelinated axons. These elements form asymmetric synapses with thin spines (see above) and dendritic trunks. There is usually a small additional band of dense membrane material beneath the postsynaptic type 2 boutons. Elements of small to medium dimensions (1-2.5 μm) have loosely packed pleomorphic vesicles and may show mitochondria, cisterns and multivesicular bodies. They may correspond to the asymmetric type of all of the above categories of axons. In addition, they participate in serial and triadic synapses, being postsynaptic to type 3 axons and presynaptic to a symmetric synaptic contact to dendrites (serial) which sometimes are also postsynaptic to the same type 3 axon (triad). Aided by USPHS Grant # NS-11631.
228 PALLIDAL NEURONS BRANCHING TO THE THALAMUS AND TO THE MIDBRAIN

229 EFFECTS OF INTRANIGRAL MICROINJECTION OF MORPHINE AND STRYCHNINE ON CAUDATE NEURONAL ACTIVITY IN THE RAT. Edward P. Fimmetty and Thr H. Chang* Department of Life Science, Indiana State University, Terre Haute, IN 47809.

Previous studies from our laboratory have implicated that sup-pression of caudate neuronal activity by intranigral microinjection of morphine (MO) may be achieved partly via a direct activation of the nigrostriatal dopaminergic pathway (Lee, Wong, and Chan, Neuropharmacology 15:571, 1976). However, the possibility that MO might also act on other nigral GABAergic neurons located in the substantia nigra (SN) zona reticulata, have been proposed to press the zona compacta (SNC) cells utilizing glycine as the inhibitory transmitter agent. Further analysis of the mechanism of suppression of activity by MO on CN activity is needed to the role of glycine in this process.

Experiments were performed on Charles River rats placed in a cylinder and the midbrain. We have undertaken a similar study in the monkey. Lesions of the nucleus accumbens (N.Acc.) produced behav-

230 ATTENUATION OF NUCLEUS ACCUMBENS INDUCED INHIBITION OF SUBSTANTIA NIGRA UNIT ACTIVITY BY BICUCULLINE

Simon J. Fung, Howard K. Strahlendorf, Jean C. Strahlendorf, and Charles D. Barnes, Department of Physiology, Texas Tech University School of Medicine, Lubbock, Texas 79430.

Lesions of the nucleus accumbens (N.Acc.) has been shown to act as a reinforcement signal in the behavioral performance of many animal species. The mechanism by which the N.Acc. inhibits or enhances the activity of nigral neurons is not fully understood.

Previous studies from our laboratory have implicated that suppression of caudate neuronal activity by intranigral microinjection of morphine (MO) may be achieved partly via a direct activation of the nigrostriatal dopaminergic pathway (Lee, Wong, and Chan, Neuropharmacology 15:571, 1976). However, the possibility that MO might also act on other nigral GABAergic neurons located in the substantia nigra (SN) zona reticulata, have been proposed to press the zona compacta (SNC) cells utilizing glycine as the inhibitory transmitter agent. Further analysis of the mechanism of suppression of activity by MO on CN activity is needed to the role of glycine in this process.

Experiments were performed on Charles River rats placed in a cylinder and the midbrain. We have undertaken a similar study in the monkey. Lesions of the nucleus accumbens (N.Acc.) produced behav-


Changes in GABA binding in substantia nigra (SN) were examined after lidocaine electrolytic lesions of striatopallidal projections 2) hemitransections anterior to SN 3) hemi transsections posterior to SN and 4) chronic administration of haloperidol or chlorpromazine. GABA was determined in a frozen and Triton X-100 treated crude synaptic fragment mitochondrial fraction membrane. Both high (Kp=20 μM) and low (Kp=500 μM) affinity binding sites were observed in SN. In contrast, hemi transactions posterior to SN resulted in a 30-40% decrease in specific GABA binding in SN. In contrast, hemi transactions posterior to SN resulted in a 30-40% decrease in specific GABA binding in SN when measured at 3 weeks postop. Chronic (8 weeks) treatment with haloperidol (1 mg/kg/day) or chlorpromazine (20 mg/kg/day) did not alter GABA receptors in caudate-putamen but caused a significant increase (35%) in GABA binding in SN. An increase in nigral GABA receptors may compensate for a decrease in the function of striatonigral dopaminergic neurons. This in turn may be an indirect effect of dopamine receptor blockade in the caudate. Such a compensatory mechanism may play a role in the development of tolerance to the neuroleptic-induced desensitization of tyrosine hydroxylase.

The decrease in GABA binding after transsections posterior to SN suggests that a major role of GABA receptors is to protect the SN from dopaminergic insults. This is consistent with the observation that GABA receptors located on projections descending from the nigra may be important for motor control. We postulate that the modification of nigral GABA receptor activity, produced by drug- or lesion-induced changes in striatopallidal neural function, may have impact not only on dopaminergic and other ascending pathways but also on descending projections from SN.
234 NEURONS IN THE RAT SUBTHALAMIC NUCLEUS Send AXON COLLABORALS TO BOTH THE SUBSTANTIA NIGRA AND GLOBUS PALlidUS. Toshii Hattori and Derek van der Kooy. Dept. Anat., Univ. of Toronto, Toronto, Ont., Canada.

The rat subthalamic nucleus (SN) is a densely packed group of deep stained (Nissl) neurons, situated immediately dorsal to the cerebral peduncle in the caudal diencephalon. Moving from rostral to caudal, the SN shines in the dorsomedial plane and expands in the mediolateral direction. Both multipolar and fusiform cells are seen in the SN, and they range from 1-25 μm in diameter. Smaller more perikaryal neurons can be found medially in the SN. The number of neurons in the SN on one side of the brain was estimated to be 9,500.

Autoradiographic anterograde transport studies have revealed that the two major projections from the SN are to the substantia nigra and globus pallidus. In the present study a retrograde fluorescent double labeling technique was used to investigate the organization of the SN neurons projecting to the substantia nigra in relation to those projecting to the globus pallidus. 0.1-0.3 μl of 1% Evans Blue, which fluoresces red, was injected into the globus pallidus and a similar quantity of 2.5% DAPI-10% Primuline was injected into the substantia nigra. After retrograde axonal transport of the two fluorescent tracers, over 90% of the SN neurons were double labeled with both Evans Blue and DAPI-Primuline. The smaller SN cells (previously proposed to be interneurons) were also double labeled, suggesting that the SN may generate disinhibitory inputs. At the level of the caudal two-thirds of the SN a relatively small number of double labeled cells was seen extending from the mass of SN cells slightly medially into the posterior-medial hypothalamus and laterally in a very thin strip to the far lateral edge of the cerebral peduncle. In conclusion, almost all SN axons bifurcate into ascending and descending branches, innervating the global pallidus and substantia nigra, respectively.


The islands of Calleja (Ical) not only have a close spatial relationship but also share some histochemical characteristics with the striatum and globus pallidus. The high iron content of the globus pallidus, we have used Perl's method to identify the pallidal component of the olfactory tubercle (Switzer and Hill, this volume). Using the same method, we describe here iron-positive, finger-like formations of the ventral pallidum that extend rostrally from the caudal polymorph zone of the olfactory tubercle. Each formation is capped by an island of Calleja. The iron-positive pallidal-like zone extends into the cell sparse cores of the Ical, including the magna and magnocellular nuclei. The neuropil around the grumae cells of the Ical is heavily laden with iron.

The stratal component of the olfactory tubercle, as revealed by acetylcholinesterase (AChE) stained preparations, is distinct from the iron-positive areas, and extends from the superficial strata through cell bridges of Heime to join the main body of the striatum. The Ical are located within this area and also contain AChE. The intimate spatial relationship with the ventral pallidum and striatum, as well as the similarities in histochemistry to both of these areas, suggest a role for the Ical in striato-pallidal functions.
236 INCREASED Dopamine receptor sensitivity after estrogen treatment. B.E. Bruksa and E.K. Silbergeld. Experimental Therapeutics Branch, NIMH, MD, Bethesda, MD 20205.

The effect of estrogen on dopaminergic neurotransmitters in the central nervous system was investigated biochemically and behaviorally. Male, adult rats were chronically estrogen treated by a single subcutaneous injection of estradiol valerate (125 μg/rat) in sesame seed oil. Six days after the estrogen treatment the rats were sacrificed, the brains rapidly removed, and the caudate nucleus was isolated, homogenized, and washed. The characteristics of the dopamine receptors were measured by a receptor binding assay for [3H]spiroperidol. Scatchard analysis of the binding curves indicated that there was a 20% increase in the density of the dopamine receptors, with no change in their apparent receptor affinity (101% of control).

Behavioral responses were measured after the injection of 6-hydroxydopamine (6-OHDA). Male rats were injected into the caudate nucleus (A: 7.9, L: 2.6, V: -0.4 mm; König and Klippel) with 20 μg 6-OHDA in 2 μl of saline containing 1% ascorbate. The rate rotated intensely (30-40 rotations/min) to the ipsilateral side when administered d-amphetamine (3 mg/kg). The rate also rotated to the contralateral side (10-15 rotations/min) when administered apomorphine (5 mg/kg). Estrogen treatment increased the duration of rotation to d-amphetamine, suggesting an increased sensitivity of dopamine receptors.

Male rats injected bilaterally with 6-OHDA were also tested for stereotypy after estrogen treatment. The rats treated with estrogen had an increased duration of stereotypy when injected with d-amphetamine (5 mg/kg). This again suggests that dopamine receptors are more sensitive after estrogen treatment.

These results indicate a direct effect of estrogen on dopamine receptor sensitivity as measured both biochemically and behaviorally. Biochemically, there is an increase in the number of dopamine receptors, and behaviorally, there is an increased sensitivity (rotation or stereotypy) to d-amphetamine treatment. These findings suggest an important interaction between estradiol and dopamine, which is of relevance to the pathogenesis of Parkinson's disease.


An imbalance in dopaminergic (DA) activity between the caudate-putamen nuclei (CPU) is widely believed to be reflected in postural asymmetry. However, although the CPU in the rat is regionally differentiated with respect to neurotransmitters, behavior, and some behaviors, the possibility that the CPU might be regionally distributed with respect to DA-induced postural asymmetry has not been explored. In addition, since prefrontal cortex receives a heavy DA innervation it might also play a role in DA-induced asymmetries. We implanted male Sprague-Dawley hooded rats intracerebrally with permanent cannulae and administered both DA (25 μg in .25 μl) and the vehicle alone (.5% saline, .25 μl) unilaterally on separate occasions. A series of sites throughout the CPU, the prefrontal cortex, and the tissue surrounding the CPU were tested. After injection the rats were placed in a circular enclosure in a sound-attenuated chamber and observed via a television monitor. The amount of time spent in an asymmetrical posture, time spent grooming on each side, number of rotations, and locomotor activity were measured. Regional differences within the CPU were found with the vehicle alone: injection of saline into the ventral CPU produced a strong ipsilateral deviation, while injection of saline into the dorsolateral-regions did not produce postural asymmetries. Injection of DA into both regions resulted in contralateral deviation, but while the absolute contralateral deviation scores for ventral injection were less than those for the medio-dorsal-region, the difference between the effect of the saline vehicle and the effect of DA injection was greater for the medio-dorsal region. Injection of DA into prefrontal cortex also induced contralateral deviation; DA injections into other areas surrounding the CPU did not produce postural asymmetries. For both the CPU and prefrontal cortex the ensuing data parallel those for postural deviation: No activity changes were produced by DA injection at any site; circling was infrequently seen.

We conclude that the CPU in the rat is regionally differentiated not only with respect to postural deviation but also with respect to other on-going activities such as grooming.


Intracellular recordings were obtained from substantia nigra (SN) and dorsal raphe nucleus (DRN) neurons with micropipettes. Injection of kainic acid into the caudate nucleus and globus pallidus of the rat caused a contralateral deviation; DA injections into other areas did not produce postural asymmetries. The regional differences within the CPU were found with the vehicle alone: injection of saline into the ventral CPU produced a strong ipsilateral deviation, while injection of saline into the dorsolateral-regions did not produce postural asymmetries. Injection of DA into both regions resulted in contralateral deviation, but while the absolute contralateral deviation scores for ventral injection were less than those for the medio-dorsal-region, the difference between the effect of the saline vehicle and the effect of DA injection was greater for the medio-dorsal region. Injection of DA into prefrontal cortex also induced contralateral deviation; DA injections into other areas surrounding the CPU did not produce postural asymmetries. For both the CPU and prefrontal cortex the ensuing data parallel those for postural deviation: No activity changes were produced by DA injection at any site; circling was infrequently seen.

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239 DECREASE OF NEOCORTICAL CHOLINE ACETYLTRANSFERASE ACTIVITY BY KAINIC ACID INJECTED INTO THE BASAL GANGLIA. Peter B. Kelly and Stanley L. Harraguen*. (SPON: M. J. Collier). Dept. of Physiol. and Biophysics, University of Southern California Sch. of Med., Los Angeles, CA 90033.

Choline acetyltransferase (CAT) activity, as a marker of cholinergic neurons has been measured in the neocortex after microinjections of kainic acid into the caudate nucleus and globus pallidus of the rat. Anterior, middle and posterior neocortical regions were dissected (Kelly & Moore, 1978, Exp. Neurol. 61, 749) and analyzed separately. Five days after injection of kainic acid (1.25 μg in 1 μl of saline) into the anterior, middle or posterior neocortex, CAT was significantly decreased in the anterior and middle neocortical regions. The amount of kainic acid spreading to the neocortex was assessed by including 0.1 μCi of [3H] kainic acid in the injection. Five minutes after the end of the injection, radioactivity in the neocortex was less than 10% of that remaining in the striatum. Direct injection of kainic acid (0.25 μg in 1 μl of saline over 100 sec) into the neocortex did not affect neocortical CAT though experiments with [3H] kainic acid showed greater cortical retention of radioactivity by this route than by intracaudate injection. Thus spread of kainic acid up the injection track is not responsible for the decrease of neocortical CAT. With smaller injections of kainic acid (0.625 μg in 0.5 μl, 0.25 μl/min) into the caudate nucleus or globus pallidus, decreases of CAT in anterior and middle neocortex were observed only after the pallidal injections. The results suggest that the direct projections to neocortex from neurons in the region of the globus pallidus, demonstrated by the horseradish peroxidase technique (Divac, 1975, Brain Research 93, 385), are cholinergic and sensitive to kainic acid.

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Antibodies prepared in rabbits against purified choline acetyltransferase (CAT) from human neonatal tissue have been employed to demonstrate CAT-containing neurons in the avian brain using a direct (PAP) immunohistochemical method. Normal human brains, obtained from various coroners' offices within 0.5-1 hr post-mortem, were removed from the skull and cut into 5 mm thick paraffin sections with 6 of 1% paraformaldehyde and 0.1% glutaraldehyde. Various brain regions were dissected out and post-fixed with 4% paraformaldehyde for 8 hr followed by washing with PBS at pH 7.4 for 15 hr in PBS for 48 hr. All steps were carried out at 2-4°C. Cryostat sectioned (20 μm) tissues were first treated with 0.05% Triton X-100 in PBS. For the PAP reaction, cryostat sections were incubated for 48 hr in PBS containing monoclonal antibodies against human CAT (1:100 dilution) for 48 hr. The secondary antibody was a rabbit anti-mouse IgG antibody (1:500 dilution) followed by a 1% AEC solution in PBS for 15 min. PBS was used for washing. APO (i.p.) was given to rats (6 mg/kg) and brains were obtained in the human spinal cord, cerebellum and hippocampus was in agreement with previous reports using other species of animals. In the neonatal area, however, at least two different kinds of positively staining cell bodies were observed. These included some large or giant multipolar cells (>25 μm) which possess a large, roughly spheric or oval dendritic trees and at one time thought to be efferent neurons but are, according to recent evidence, very probably interneurons. Small multipolar cells (<10 μm) with round shaped somata were also stained. The globus pallidus contained a high density of long positive fibers and a rather scattered population of large multipolar neurons.

BASAL GANGLIA


In pigeons, equal parts mixtures of 3H-leucine and 3H-proline were placed in the dorsomedial striatum augmentatum (PA) or lateral hypothalamus (LPO) of the pigeon. Using autoradiographic and morphometric analysis, revealed heavy silver grain accumulations over the central and dorsal portions of the pars compacta of the substantia nigra tegmentum. These procedures are confirmed by ventrally placed dorsal pars disserratae at dorsals (TPD) of the nucleus pedunculo pontinus.

In prior studies PF and NP appear not to receive monoaminergic innervation. Taken together, these results indicate the existence of reciprocal connections between neurons of the avian paleostriatum and midbrain tegmentum comparable to those existing in mammalian forms between the striatum and substantia nigra area.

INTERCONNECTIONS OF AVIAN PALSSTRIATUM AND MI DBRAIN TEGMENTUM. Cheryl A. Kitt and Steven E. Brauch. Dept. of Psychol., Univ. of Maryland, College Park, Md.

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INTERCONNECTIONS OF AVIAN PALSSTRIATUM AND MI DBRAIN TEGMENTUM. Cheryl A. Kitt and Steven E. Brauch. Dept. of Psychol., Univ. of Maryland, College Park, Md.
The posterior intralaminar thalamic, consisting of centrum medianum (CM), n. parafascicularis (PF) and posterior n. centralis lateralis, is the source of a major projection to the caudate nucleus in the cat. Thus, an obvious question is how these nuclei differentiate in size and location. The stereotaxic coordinates defining these structures cannot be used with confidence in studies of the thalamo-striatal projection system.

To resolve this problem, we used HRP (7MB) method to define the intralaminar projection to the caudate-putamen. Large injections of HRP were used. The cortical, caudate and small lenticular HRP deposits were used to determine its topographic organization. Clusters of retrogradely labeled cells were found surrounding the habenulo-interpeduncular tract (retroflex bundle) dorsally, ventrally, and laterally, but not medially.

The projection was topographically organized. This delineation of CM agrees with that of the rat stereotaxic atlas of Albe-Fessard et al., but differs from that of the atlas of König and Klippel.

### Cholinceptive Neurons in the Isolated Caudate

- **Heterogeneity Within the Caudate Nucleus of the Cat:** Bioclinical and Histologically Investigations. John Lehmann, R. Fibiger and A. Parent. Division of Neurological Sciences, University of British Columbia, Vancouver B. C., 1965, and (A.P.) Dpartment d'Anatomie, Universite Laval, Quebec.

- **Cholinoceptive Neurons in the Isolated Caudate:** Support of the Medical Research Council.
QUANTIFICATION OF DENDRITIC MORPHOLOGY IN THE DEVELOPING CAUDATE NUCLEUS OF THE CAT. J.P. McAllister*, R.W. Bradford*, M.S. Levine C.D. Hul and A.M. AdesNI, Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA, 90024.

As the continuation of a series of investigations designed to quantify synaptic development of the cerebral cortex, the present study has examined the dendritic growth of spiny neurons in the kitten caudate nucleus. Tissue from 3, 10, 19 and 114 day old kittens was injected with the rapid Golgi method. Neurons from each age group were drawn using a camera lucida attachment and subsequently three-dimensional analysis performed with the aid of a PDP 11/40 computer system. In this way, accurate measurement was obtained on a variety of dendritic parameters. Results from the four age groups were compared to determine how the dendritic morphology of the spiny neurons changes with development. Preliminary analyses from 66 neurons (3 day = 15; 10 day = 60pm in the 3, 10 and 19 day groups to 85pm in the 114 day group. Similarly, the average dendritic length measures 494, 592, 440 and 286um at 3, 10, 19 and 114 days, respectively. The lower values for average dendritic length and number of branches per dendrite (7.7) at 19 days may reflect impregnation differences in that all cells at that age were measured from a single animal. Nevertheless, the maximum values, which should compensate for dendrites which may appear prematurely shortened because they pass out of the section, were still significantly higher than at 3, 60pm and 303um at 3, 10, 19 and 114 days, respectively. Such linear growth occurs distal to the primary dendrite, because the lengths of the first branch do not increase significantly. In contrast, the total branch lengths comprising an entire dendritic field are relatively uniform, ranging from 2650 to 2990um for the early age groups to about 3300um at 114 days. This may correlate with the most striking finding, that the total number of dendrites per neuron appears to increase with age. At 3, 10 and 19 days there are 6.1, 5.3 and 6.2 dendrites per neuron, respectively. This compares to 4.4 dendrites per neuron at 114 days. Loss of dendrites with age may be due to fusion of basal membranes at the point of origin to provide a common primary stem for two or more main secondary branches.

Supported by USPHS Grants Nos. HD-05998, HD-04512, VR-5156.

THE EFFECTS OF AGING ON NEURON "CELL ISLANDS" IN THE MOUSE NEOSTRIATUM, Patricia L. Mensah. Department of Anatomy, University of Southern California School of Medicine, Los Angeles, CA.

A number of studies have shown that neuronal aggregates or islands characterize the vertebrate neostriatum. To evaluate the possibility that these cellular territories change with age, the brains of C57B1/6J mice 4, 10, and 114 months of age were fixed in Carney's solution and embedded in paraffin. Five micron coronal sections through the head of the caudate-putamen nucleus (the region rostral to the crossing of the anterior commissure), were stained with cresyl violet. Five sections from each animal were analyzed. The mediolateral extent of each neuronal domain was determined. Of the domains defined at 4 months, a large domain extends from the midbrain (Parent, J. Anat., 114:379, 1973). The turtle olfactory tubercle shows rudimentary lamination of neuronal elements and in its depths is continuous with the underlying striatum. Extracellular recordings have been made in the olfactory tubercle following electrical stimulation in the following areas: olfactory nerve (ON), lateral olfactory tract (LOT), lateral forebrain bundle at the base of the striatum (LFB) and midbrain. Stimulation of ON, LOT, and LFB elicited a small negative field potential within 100 μm of the surface, and, in deeper layers, a large positive field potential with a similar time course. Extracellularly recorded single units which responded to ON stimulation were found to a depth of 600 μm from the surface, i.e., well into the striatum. These cells frequently responded to LOT and LFB stimulation as well. The effects of various combinations of paired volleys were examined and prominent effects was a long-latency facilitation of LOT and LFB test volleys following conditioning volleys in the same pathway. Another finding was a long-lasting suppression of ON test volleys following LOT conditioning.

Intracellular recordings have revealed impulse responses to all four stimulation sites. Several cells demonstrated brief EPSPs which gave rise to action potentials, followed by long-lasting IPSPs of several hundred msec duration. The effects of dopamine agonists and antagonists on the response of the EPSP-IPSP sequences are currently under investigation.


The lateral habenular nucleus (LHB) receives projections from the entopeduncular nucleus (entopeduncular nucleus of globus pallidus of the cat) and the substantia nigra. The habenula is involved in the regulatory control of both motor and sensory systems. Its role in these functions is thought to be some degree of segregation of afferent and efferent LHB pathways. Anterograde and retrograde transport studies have shown the LHB receives inputs from various areas, the degree of segregation of afferent and efferent LHB pathways. Anterograde and retrograde transport studies have shown the LHB receives inputs from various areas, the degree of segregation of afferent and efferent LHB pathways. Anterograde and retrograde transport studies have shown the LHB receives inputs from various areas, the degree of segregation of afferent and efferent LHB pathways. Anterograde and retrograde transport studies have shown the LHB receives inputs from various areas, the degree of segregation of afferent and efferent LHB pathways.
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Recurrent inhibition has been demonstrated in rat caudato-putamen following direct stimulation, during intracellular recording, of a single motoneuron. Action potentials were trig-gered by depolarizing square wave current pulses (2-5 msec; 0.5-5mA) conditioned a test EPSP evoked from stimulation of substantia nigra (SN). Reduction of the test EPSP by up to 35% of inhibition localized in the dendrites, occurred at interstimulus intervals of less than 20 msec. In nearly all cases where suppression of the test EPSP was observed, a corresponding hyperpolarizing potential was seen at the recording site. Time to peak of the test EPSP was not altered but its rate of decay was markedly increased. Neurons showed depolarizing following action EPSP often deepened. Reduction of the test EPSP by up to 53% was observed and was always less than that seen for inhibition resulting from solely extrinsic stimulation, as in double SN stimulation. To eliminate the possibility that the reduction of the test EPSP was due to an increase in conductance resulting from AP currents, two tests were performed. 1) When depolarizing pulses (250-350 msec) were injected triggering only a single AP, the depolarizing plateau following the AP remained flat, indicating no membrane conductance change. 2) If the conditioning AP was followed by a train of hyperpolarizing current pulses (1nA; 5.0 msec), there was no measurable conductance change. Neurons injected with HRP subsequent to testing for recurrent inhibition were recovered and identified as medium spiny neurons. (Supported by USPHS Grant NS 14886 and NIBRS RG 50772-04.)

The neurographic evidence suggests that the cortico-striate projection in cat's motor cortex is organized in a complex pattern in which both grain-dense and grain-sparse areas can be recognized within the field of distribution of individual cortical areas. Since the grain-sparse zones and grain-dense zones have the same dimensions as inhomogeneities in striatal acetylcholinesterase (ca. 500 nm dia.), it seems possible that the two patterns might be related. We have therefore compared them directly in the caudate nucleus of the cat.

Injections of 8-aminooacid or 8-selanthione were made into the pericruciate cortex of three cats. Up to 1 ml of label was deposited in multiple closely spaced injections and the resulting injection sites involved parts of areas 4, 6 and surrounding cortical areas. Most portions were prepared for autoradiography, but at intervals sections were instead prepared by the acetylthiocholinesterase (AChE) method of Cueneser-Jensen and Blackstad. Both processing techniques demonstrated marked inhomogenization in the striatum. In the autoradiograms the caudate nucleus was massively labelled on both sides in a patterned manner confirming earlier accounts; in the AChE-sections irregularly shaped areas of low activity appeared against a matrix of dark stain. Auto- radiograms were lined up with neighboring sections stained for cholinesterase. It was clear that while the inhomogeneities in grain-distribution in the autoradiograms were by no means always aligned with the zones of low AChE activity, there was a matrix: sometimes the borders of the pale AChE zones corresponded to the borders of the pale AChE zones, sometimes grain-sparse and enzyme-poor zones were aligned. The grain-sparse areas corresponded to the cholinesterase-negative zones appearing in the ventral half of the caudate nucleus both ipsilaterally (3 cases) and contralaterally (1 case). By contrast, instances of "filling in" of AChE-poor sections by grains corresponding to surrounding autoradiograms were seen in the dorsal part of the caudate nucleus ipsilaterally (2 cases) and contralaterally (1 case). These last named alignments were especially prominent in one case (P76) in which the cortical injection was centered in area 6.

We do not know how extensive such systematic correspondences may be for other cortical afferents. In the fronto-striate projection quantitative analysis is difficult for technical reasons: in both the AChE staining and the autoradiography one must balance between prolonged processing that could mask some of the grain-sparse areas or grain-sparse zones, and brief processing that might fail to demonstrate the inhomogeneities adequately. Supported by NSF 76-10554-NBS and NIH-1-R01-ET02886-01.


Microinjections of the neurotoxin, kainic acid (KA), into the striatum of rats produce biochemical, anatomical and behavioral changes similar to those seen in patients with Huntington's disease (HD)(1,2). The present study examined the effects of KAL rats showed clonic convulsions. Catalepsy effect, compared to other non-striatal damaged neurological conditions, is compatible with the encoding of stimulus location during ingestion-related movements and subsequently for sensory responsiveness.

Three populations of cells showed ingestion-related changes in activity. The most frequently encountered type (25% of sampled cells) showed firing rate changes that were time-locked to milk delivery, improving somatosensory or gustatory sensitivity. Response magnitude was unrelated to the topography of ingestion-related movements; indeed, unit responses persisted in the absence of any motoric response to the milk. Current work is investigating the possible involvement of these cells in attentional aspects of behavior.

The second type (15% of sampled cells) was characterized by a high degree of somatosensory responsiveness. Light tactile stimulation was optimal for effecting activity changes. Receptive field sizes were moderate (eg. front of lip) to large (entire face) and always included the perioral region. Sensitivities of receptive fields were increased in firing in these cells. Receptive fields were contra-lateral, perioral, and located far posterior of the area contacted by the drinking tube. These response characteristics are compatible with the encoding of stimulus location during movement and suggest possible targeting functions.

These data underscore the involvement of the basal ganglia in oral-ingestive behavior. However, the nature of this involve-ment remains unclear, particularly in view of the absence of pure movement-related activity with potential involvement of trigeminal afferents upon caudate neurons emphasizes analysis of sensory processing as an advantageous approach to an understanding of basal ganglia function.
A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE DEVELOPING CAUDATE NUCLEUS which are characteristic of immature neurons, were noted at the maturation occurs during the first postnatal week. The somata were examined for maturational changes in somatic size, dendritic ties approached those seen in the adult while the density in the 19-20 day old material had

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In Nissl-stained, parasagittal sections of rat brain the globus pallidus (GP) seems to extend rostro-ventrally in a cusp-like formation into the anterior rostral part of the head of CN. After 5-8 days, bilateral microinjection of Pi or NaCl was performed but 10 min after bilateral microinjection of Pi or NaCl. Similar samplings were obtained in the following 40-80 min. After the Pi microinjections the first and second; sign test). The described results further support the suggestion that the Cr usually acts in a manner which produces inhibition both electrophysiologically and behaviorally.
SINGLE DORSAL RAPHE NEURONS PROJECTING AXON COLLATERALS TO BOTH THE SUBSTANTIA NIGRA AND CAUDATE-PUTAMEN IN RAT. Derek van der Kooy and Toshi Rattani. Department of Anatomy University of Toronto, Toronto, Ontario, Canada.

The serotonin containing neurons of the dorsal raphe (DR) nucleus are known to have widespread forebrain connections. Single DR cells have been shown to innervate dorsal brain areas through collateral axons. Recent autoradiographic anterograde transport reports have detailed the projections of the DR to the substantia nigra. In the present retrograde fluorescent double labeling study investigated the organization of the raphe cells projecting to the substantia nigra in relation to those projecting the caudate-putamen in rat. 0.2 - 0.4 uL of 10% Evans Blue was injected into the caudate-putamen over two needle penetrations. 0.1 - 0.3 uL of 2.5% DAPI-Primuline, which fluoresces blue, was injected into the substantia nigra lateral to the major bundle of serotonergic axons coursing in the ventral tegmental area. Neurons retrogradely labeled with Evans Blue were seen throughout the DR, whereas neurons labeled with DAPI-Primuline were restricted to the dorsal cluster portion of the DR. Neurons labeled with E. Blue were more numerous than those labeled with DAPI-Primuline, which fluoresces blue, in the substantia nigra lateral to the major bundle of serotonergic axons coursing in the ventral tegmental area. Neurons retrogradely labeled with Evans Blue were seen throughout the DR, whereas neurons labeled with DAPI-Primuline were restricted to the dorsal cluster portion of the DR. Even in this dorsal cluster, Evans Blue labeled cells outnumbered the DAPI-Primuline labeled cells. However, the most important finding was that 70% to 90% of the DAPI-Primuline labeled DR cells were double labeled with Evans Blue. The labeled DR cells were both fusiform and multipolar in shape. The average size of the labeled DR cells was approximately 18 μm in diameter, similar in size to the DR neurons supposed to contain serotonin. Smaller size DR neurons (average of approx. 13 μm) were less likely to be labeled. The ascending raphe axons can be separated into six separate bundles. Differences in size appear to innervate the substantia nigra and caudate-putamen. The present results suggest that single dorsal raphe cells send axon collaterals into more than one of the ascending bundle. In conclusion, two interrelated portions of the basal ganglia (caudate-putamen and substantia nigra) appear further related by virtue of their innervation from the same neurons in the dorsal portion of the DR.

TESTOSTERONE AND 17β-ESTRADIOL LEVELS IN ACAUDATE, AFRONTAL AND BASAL GANGLIA OF INTACT AND AUXOVISUAL MANIPULATED RATS. W. Kooy and Toshi Hattori. Department of Anatomy University of Toronto, Toronto, Ontario, Canada.

We have previously defined the behavioral changes which follow extensive caudate nuclei lesions in cats and kittens. A characteristic of caudateablated male cats is the appearance, immediately after recovery from anesthesia, of a stereotypy-like behavior to stimulation of the perigenital region which includes lordosis, tail deviation, hind paw treading and, occasionally, vocalization. We present experiments designed to test if there is an endocrinological basis for this behavior. It has been postulated that the striatum may be involved in endocrine control (Barbeau, 1970, 1973). Plasma levels of testosterone and 17α-estradiol (Schill, et al., in press) were measured by radioimmunoassay in intact (N=7), caudate-resected (N=7; median removal 84%), and sham-resected (N=4; all tissue in front of A 22 removed) and operated control (N=4; 2 with a large septal lesion and 2 with hemispherectomy). Plasma samples (3-5 ml) were taken at two day intervals from restrained cats over a two week period for a total of 10 samples per subject. The data analysis was on the daily medians for each group, subject medians across samples and grand medians for daily and subject medians. Intact values for both testosterone (md 6.7, range 2.69-93 ng/ml) and estradiol (md 15.4, range 10.0-19.6 pg/ml) agreed with values from other labs. A two-way ANOVA showed a significant group effect for both median testosterone (p<.05) and 17α-estradiol (p<.001). Comparisons of the group medians (Mann-Whitney U) showed that both acuates (p<.05) and frontalats (p<.05) had significantly reduced levels of testosterone than intact animals. Comparisons for 17α-estradiol showed frontalats to be significantly higher than either intact animals (p<.001) or caudates (p<.001). While the operated controls were significantly lower (p<.001) than the other groups. Spearman rank correlations between magnitude of the caudate lesion and plasma levels were not significant for either testosterone (r=0.34, p<.05) or 17α-estradiol (r=0.0, p>1.0). However, a similar correlation of lesion and estrus-like behavior was significant (r=0.84, p<.005) and the behavior was suppressed in caudates (p<.001). These data suggest that decreased plasma levels of testosterone are not specific to caudate ablation. In fact, this is evidence of definitively a hormonally mediated estrus-like behavior (acuate) and of an increase in estrogens without detectable sexual changes (frontal). We therefore hypothesis that the estrus-like behavior seen in acuates is probably due to changes in somatosensory responsiveness and in "affect" that we have reported and not to shifts in their endocrinological status. (USPHS Grants HD-05598 and HD-04612).


We have previously defined the behavioral changes which follow extensive caudate nuclei lesions in cats and kittens. A characteristic of caudate- ablated male cats is the appearance, immediately after recovery from anesthesia, of a stereotypy-like behavior to stimulation of the perigenital region which includes lordosis, tail deviation, hind paw treading and, occasionally, vocalization. We present experiments designed to test if there is an endocrinological basis for this behavior. It has been postulated that the striatum may be involved in endocrine control (Barbeau, 1970, 1973). Plasma levels of testosterone and 17α-estradiol (Schill, et al., in press) were measured by radioimmunoassay in intact (N=7), caudate-resected (N=7; median removal 84%), and sham-resected (N=4; all tissue in front of A 22 removed) and operated control (N=4; 2 with a large septal lesion and 2 with hemispherectomy). Plasma samples (3-5 ml) were taken at two day intervals from restrained cats over a two week period for a total of 10 samples per subject. The data analysis was on the daily medians for each group, subject medians across samples and grand medians for daily and subject medians. Intact values for both testosterone (md 6.7, range 2.6-9-3 ng/ml) and estradiol (md 15.4, range 10.0-19.6 pg/ml) agreed with values from other labs. A two-way ANOVA showed a significant group effect for both median testosterone (p<.05) and 17α-estradiol (p<.001). Comparisons of the group medians (Mann-Whitney U) showed that both acuates (p<.05) and frontalats (p<.05) had significantly reduced levels of testosterone than intact animals. Comparisons for 17α-estradiol showed frontalats to be significantly higher than either intact animals (p<.001) or caudates (p<.001). While the operated controls were significantly lower (p<.001) than the other groups. Spearman rank correlations between magnitude of the caudate lesion and plasma levels were not significant for either testosterone (r=0.34, p<.05) or 17α-estradiol (r=0.0, p>1.0). However, a similar correlation of lesion and estrus-like behavior was significant (r=0.84, p<.005) and the behavior was suppressed in caudates (p<.001). These data suggest that decreased plasma levels of testosterone are not specific to caudate ablation. In fact, this is evidence of definitively a hormonally mediated estrus-like behavior (acuate) and of an increase in estrogens without detectable sexual changes (frontal). We therefore hypothesis that the estrus-like behavior seen in acuates is probably due to changes in somatosensory responsiveness and in "affect" that we have reported and not to shifts in their endocrinological status. (USPHS Grants HD-05598 and HD-04612).


Immunohistochemical localization of GABA-T in the striatum is difficult to discern. This same problem has been reported for Acetylcholinesterase staining in the basal ganglia and was resolved by using the DFP pharmacohistochemical technique (Butcher, 1975). Recently, two specific and irreversible GABA-T inhibitors (EOS & Gabaculine) have become available, and we reasoned that they could be used to resolve the localization of GABA-T in the basal ganglia. Adult Wistar rats received intracranial injections of EOS (25mg/kg) and were sacrificed and processed with our modified GABA-T histochemical method at various survival times. Forty-five minutes after the injection of EOS, no striatal neurons staining for GABA-T could be observed. By four hours some neurons could be observed which stained for GABA-T, indicating that they had resynthesized the enzyme. Eight hours after the injection, the regeneration of GABA-T staining was almost complete. These results indicate that this pharmacohistochemical procedure for GABA-T may be a useful tool for the localization of GABA systems in the basal ganglia, and other areas of the central nervous system.

Supported by the Medical Research Council.
Electrophysiological and Biochemical Examination of the Cholinergic System in Rat Neostriatal Slices. *Dolly H. Weiller*, Urich *Hugel*, Il-In Su, Donald J. *Jenden*, Dept. Pharmacol. and Neuro., UCLA School of Medicine, Los Angeles, CA 90024 & Max-Planck Inst. Brain Res., Frankfurt, Federal Republic Germany.

Neostriatal slices were superfused with oxygenated artificial cerebrospinal fluid (65.5 molar). Cholinergic and serotonergic (5-HT) receptors were stimulated with acetylcholine chloride and serotonin, respectively. The effects of these drugs were measured in terms of inhibition of EPSP and IPSP, respectively. The results indicated that acetylcholine and serotonin had similar effects on the release of excitatory and inhibitory transmitters, respectively. These findings suggest that both acetylcholine and serotonin may play a role in the regulation of neuronal activity in the neostriatum.

Supported by HD-05958 and the Scott Fund.
ACETYLCHOLINESTERASE NEURONS IN THE RAT CAUDATE-PUTAMEN COMPLEX:
CHARACTERIZATION OF SOMATA TYPES AND STATISTICAL RELATIONSHIPS TO
ONE ANOTHER AND TO THE TOTAL POPULATION OF STRIATAL CELL BODIES.
Nancy J. Wolf*1 and Larry L. Butcher.1,2 Dept. Psych.1 and
Brain Res. Inst.2, UCLA, Los Angeles, CA 90024.
Using a pharmaco-histochemical regimen for acetylcholinesterase
(AChE, EC 3.1.1.7) that demonstrates the enzyme in neuronal soma-
ta and their proximal processes to a degree not possible with
other protocols (Butcher & Bilezikjian, Eur. J. Pharmacol., 1975,
34, 115-125), we examined the morphologies, organization, and
staining patterns of AChE-containing neurons at 9 different ro-
stral-caudal levels of the rat caudate-putamen nucleus. At each
of these levels additional analyses were performed in the dorso-
lateral, dorsomedial, ventromedial, and ventrolateral quadrants.
As described in previous publications from this laboratory, three
broad categories of AChE-containing neurons could be discerned on
the basis of soma dimensions. Of these, the Type I cells (max-
imum soma extent = 8-12µm) were most numerous, representing 50-
65% of the total population of AChE striatal neurons. The Type I
cells were more numerous at rostral and caudal levels than in
medial portions of the nucleus. Over 70% of Type I neurons had
oval cell bodies, and 75% stained lightly for AChE. Type II neu-
rons (maximum soma extent = 13-23µm) could be further divided into
two subcategories. Type IIa cells (maximum soma extent = 13-15
µm) represented 11% of the total number of AChE neurons in the
striatum; Type IIb cells (maximum soma extent = 16-23µm) repres-
ented 27%. Most Type IIa and IIb neurons were oval. The Type
IIa somata stained lightly or with medium intensity, the number
of cell bodies in each intensity category being essentially equal.
Over 80% of Type IIb soma stained with medium intensity. Type
III cells (maximum soma extent = 24-30µm or greater), represent-
ing roughly 5% of the total number of striatal AChE neurons, were
primarily fusiform and stained darkly or with medium intensity.
The proportion of the three types of AChE somata to one another
was approximately the same at all rostral-caudal levels of the
caudate-putamen complex. Type I cells appeared slightly more
concentrated in ventral portions of the nucleus, however.
In brain sections processed first for AChE (pharmaco-histochemi-
cal regimen) and then counterstained with thionin, it was found
that AChE-containing neurons represented only approximately 3% of
the total number of neurons in the caudate-putamen nucleus. Yet
these neurons presumably account for all of the AChE in a struc-
ture possessing one of the highest concentrations of acetylcholine
(Butcher et al., J. Microwave Power, 1976, 11, 61-65) and
choline acetyltransferase in the mammalian brain. [Support: USPHS
NS 10928 to L.L.B.].
BRAIN METABOLISM AND NUTRITION
EFFECT OF ACUTE ADMINISTRATION OF NEUTRAL AND OTHER AMINO ACIDS ON CATECHOLAMINE (CA) METABOLISM


We have previously shown that tyrosine loading raises urinary CA and that this effect is blocked by pretreatment with a peripheral decarboxylase inhibitor, carbidopa. This finding suggests that TVR acts by increasing the saturation of the enzyme tyrosine hydroxylase, thereby enhancing CA synthesis in the sympatheticadrenal system. However, mechanisms by which central hypothalamic action via inhibition of sympathetic outflow, (ii) inhibition of CA degradation (III) a non-specific stress effect, causing CA release. The present study was designed to characterize the specificity of TVR effect and its biochemical consequences. Neutral amino acids (NAA) (TRF, VAL, ISOLEU, LEU, TYR) and non-neutral amino acids (GLU,ALA, lys, ARG) were administered (at concentrations equimolar with 200 mg/kg of tyrosine) to overnight fasted rats. Urines (collected for a 3 hr. period), blood, brain, and adrenals were analyzed for CA, ty, and NOSIGS-SO.

TVB caused 2-3 fold increase in tissue and blood TVB and urinary CA whereas NAA depressed these by 10-37%. GLU and ALA had no effect while LYS and ARG increased urinary CA by 10-30%. Serum TVB was highly correlated with urinary CA (r=.86). Except for TVR, changes in blood glucose, blood pressure, and heart rate were significant. TTVB caused a significant increase in urinary CA. TRT potentiated the effects of blood, brain, adrenal and urinary DA. TRR alone significantly raised urinary DA (P<.005). In adrenalectomized rats TTVB enhanced urinary DA and NE but had no effect on E. (Basal levels of DA and NE were similar in adrenalectomized and control rats.) These results show that (a) TTVB is unique in causing major increase in urinary CA; its effect is not mimicked by related NAA; (b) changes in serum TVB are reflected by parallel changes in urinary CA; (c) TRT into peripheral tissues seems to be subject to competition by NAA; however levels of NAA needed to compete are probably much higher. This study provides further evidence that availability of the amino acid precursor tyrosine affects peripheral CA synthesis and release. (These studies were supported in part by a grant from the USPHS.)

THE EFFECT OF CHRONIC MIDDLE CEREBRAL ARTERY OCCLUSION ON LOCAL BLOOD VOLUME AND CYTOCHROME a3 REDOX LEVELS IN RAT BRAIN. R.J. Bergquist and A. SylVis. Department of Physiology, Duke University Medical Center, Durham, N.C. 27710 and Division of Neuropsychiatry, M.I.T., Cambridge, Mass.

The resting local blood volume and reduction/oxidation (redox) level of cytochrome a3 was monitored in the brain region over a chronically occluded middle cerebral artery (MCA) on the ipsilateral side and compared with the opposite side (contra-lateral). The vascular and metabolic responses to alterations in the fraction of oxygen in arterial blood gases and blood pressure were performed over the lateral, superior surface just posterior to the coronal suture. Arterial blood gases and blood pressure were monitored (at concentrations equimolar with 200 mg/kg of tyrosine) to overnight fasted rats. Urines (collected for a 3 hr. period), blood, brain, and adrenals were analyzed for CA, ty, and NOSIGS-SO.

TVB caused 2-3 fold increase in tissue and blood TVB and urinary CA whereas NAA depressed these by 10-37%. GLU and ALA had no effect while LYS and ARG increased urinary CA by 10-37%. Serum TVB was highly correlated with urinary CA (r=.86). Except for TVR, changes in blood glucose, blood pressure, and heart rate were significant. TTVB caused a significant increase in urinary CA. TRT potentiated the effects of blood, brain, adrenal and urinary DA. TRR alone significantly raised urinary DA (P<.005). In adrenalectomized rats TTVB enhanced urinary DA and NE but had no effect on E. (Basal levels of DA and NE were similar in adrenalectomized and control rats.) These results show that (a) TTVB is unique in causing major increase in urinary CA; its effect is not mimicked by related NAA; (b) changes in serum TVB are reflected by parallel changes in urinary CA; (c) TRT into peripheral tissues seems to be subject to competition by NAA; however levels of NAA needed to compete are probably much higher. This study provides further evidence that availability of the amino acid precursor tyrosine affects peripheral CA synthesis and release. (These studies were supported in part by a grant from the USPHS.)

CHANGES IN CORTICAL OXIDATIVE METABOLISM ELECTRIC ACTIVITY WITH ALTERED BRAIN PERFUSION PRESSURE. George A. Austin, Ronald E. Nuss and Joseph Willey. University of Kentucky, College of Medicine, Section of Neurosurgery and Department of Physiology, Loma Linda University, School of Medicine, Loma Linda, CA 92350

Brain perfusion pressure is the difference between the diastolic blood pressure and intracranial pressure. In the case of the open skull, intracranial pressure is normally close to zero and perfusion pressure equals local or diffuse arterial blood pressure. We have studied cortical oxidative metabolism in cats under N2O/O2 anesthetic in a 2:1 ratio, where the perfusion pressure was altered locally by (i) bilateral common carotid occlusion; (ii) unilateral common carotid occlusion; and, (iii) pial artery compression. Perfusion pressure (PP) was altered diffusely by a) Intravenous Arfonad (lowered BP); b) Intravenous Ephedrin (raised BP); or c) arterial bleeding (lowered BP). In general, these techniques caused a change in cortical PO2 (P02), which was proportional to the change in mean BP. The redox state of NADH and Cyta3 was monitored by non-invasive optical techniques. CORTICAL electric activity was monitored with bipolar Ag-AgCl electrodes and analyzed in the frequency domain by a Fast Fourier Transform technique. Usually, decreased PP produced decreases in TCA, and this was accompanied by a decrease in mean BP. Reducing the FIO2 prior to lowering the perfusion pressure accelerated the reduction of NADH and Cyta3, as well as prolonging the recovery period. Cortical electric activity, after 5-30 sec. of decreased PP, was initially increased in amplitude between 0.5 and 40 Hz. These studies show that the changes in urinary CA whereas NAA depressed these by 10-37%. GLU and ALA had no effect while LYS and ARG increased urinary CA by 10-37%. Serum TVB was highly correlated with urinary CA (r=.86). Except for TVR, changes in blood glucose, blood pressure, and heart rate were significant. TTVB caused a significant increase in urinary CA. TRT potentiated the effects of blood, brain, adrenal and urinary DA. TRR alone significantly raised urinary DA (P<.005). In adrenalectomized rats TTVB enhanced urinary DA and NE but had no effect on E. (Basal levels of DA and NE were similar in adrenalectomized and control rats.) These results show that (a) TTVB is unique in causing major increase in urinary CA; its effect is not mimicked by related NAA; (b) changes in serum TVB are reflected by parallel changes in urinary CA; (c) TRT into peripheral tissues seems to be subject to competition by NAA; however levels of NAA needed to compete are probably much higher. This study provides further evidence that availability of the amino acid precursor tyrosine affects peripheral CA synthesis and release. (These studies were supported in part by a grant from the USPHS.)

This work was supported by NIA Grant AG 00517 (A.L.S.).
CEREBRAL REGIONAL O2 CONSUMPTION AND SUPPLY IN CATS.

Ellen Buchweitz, Harvey R. Weiss, and Arayinda K. Sinha


The present study represents the first quantitative measurement of O2 consumption and supply on a regional basis in the brain through the application of a new microspectrophotometric method. Twelve cats were tranquilized with ketamine and then anesthetized with α-chloralose. Artificial respiration was begun, and a left thoracotomy performed at the 5th intercostal space. Left atrial and femoral artery catheters were inserted. Heart rate, blood pressure, arterial and superior sagittal sinus blood samples were obtained. 141-Ce microspheres (15 ± 3 µ) were injected into the left atrium and cerebral blood flow determined by the reference sample method. The cats' heads were quickly cut in 2 parts and frozen in liquid N2. Ten different regions of the frozen brain were examined. Arterial and venous O2 saturations were measured in each region by examination of vessels in 30 µ frozen sections with a Zeiss microspectrophotometer. O2 consumption was determined as the product of flow and O2 extraction. Blood pressure, heart rate, and blood gas values were within the normal range. Flow values ranged from 36 ± 9 ml/min/100g (Mean ± S.E.) in the medulla to 60 ± 20 ml/min/100g in the hippocampus. The highest O2 extraction was noted in the thalamus (6.4 ± 1.0 ml O2/100ml) and the lowest O2 extraction in the anterior cortex (4.9 ± 0.5 ml O2/100 ml). O2 consumption ranged from 1.4 ± 0.4 ml O2/min/100g in the medulla to 2.8 ± 0.8 in the thalamus. The lowest ratio of oxygen supply to demand was 2.76 ± .45 in the thalamus. Supply of O2 to the brain regions studied was more than adequate to meet demand throughout the brain despite a twofold range in regional O2 consumption. This new method for the determination of O2 supply to the brain is applicable to many experimental conditions.

Supported by grants from NIH and other sources.

BRAIN METABOLISM AND NUTRITION


We have demonstrated previously that polynsaturated fatty acids (PUFAs) induce rat brain cortical edema in vitro (Science 200:358, 1978). Edematous cortex is characterized by increased water content, lactate production and intracellular Na+, and decreased extracellular space and intracellular K+. The present study deals with brain edema induced by arachidonic acid (C20:4) and the effects thereon of bovine serum albumin (BSA). Cortical edema induced by arachidionate was dependent on the duration of incubation. Induction of brain edema approached a plateau after a 30 min incubation in 0.5 mM arachidionate which was followed by a 60 min additional incubation in Krebs-Ringer control medium or in BSA (0.1%). Co-incubation of arachidionate (Ara) with BSA at a molar ratio of 5 (Ara/BSA) or less greatly inhibited the arachidionate-induced effects. Cortical edema increased as the molar ratio increased above 5. However, the swelling was not reversible by BSA, although the post-incubation of BSA released 44% of incorporated [3H]arachidonic acid in cortical slices. [3H]-arachidonic acid transport was completely abolished by 0.1 mM BSA and partially inhibited by exogenous arachidonate. We conclude that brain edema induced by arachidonate requires that free or unbound exogenous arachidonate be transported into cortical slices. It does not appear to be a surface effect. The molar ratio of Ara/BSA plays a critical role in the binding and the transport of arachidonate in isolated rat brain cortex. Supported by NIH Grant NS 14543.

INTRAVENTRICULAR INFUSIONS OF GLYCEROL PRODUCE BODY WEIGHT LOSS IN FEMALE RATS. Barbara J. Collins* and John D. Davis (SPON. Ernest W. Kent). Dept. Psychol., Univ. of Illinois, Chicago, Ill. 60680.

It has been previously determined by Wirtshafter and Davis (Science 198:1271, 1977) that subcutaneous injections of glycerol (40 mg/kg 4 times daily) produced body weight loss during the period of injection. However, subcutaneous injection of glycerol was not found to produce an effect in female rats. In the present experiment, injection of small amounts of glycerol into the third ventricle was shown to produce a decrease in food intake and body weight in females, as did similar injections in males previously reported by Davis and Wirtshafter (Neurosciences Abstracts, no. 525, 1978). Body weight and food and water intake were measured twice daily in female rats before, during, and after 11 days of continuous infusion of glycerol into the third ventricle. Vaginal smears were taken concurrently with above measurements to determine the stage of the estrus cycle. In uncamouflaged rats, subcutaneously implanted Alzet osmotic minipumps were connected to a stainless steel ventricular cannula by a polyethylene tubing. Glycerol was delivered at a rate of 27 µg/hr in a volume of 0.3 µl/hr.

During the period of infusion body weight decreased significantly, but increased after infusion was stopped. Food intake also showed an overall decrease during infusion. However, the normal rhythm of food intake in the female rat, which demonstrated a decrease in intake on proestrus and an increase during estrus and diestrus, was not disrupted by glycerol infusions.

Inhibition of protein synthesis during cerebral ischemia and delayed or incomplete recovery of synthesis in the post-ischemic recirculation phase could contribute to irreversible cell damage after stroke. We induced hemispheric ischemia in conscious rats by occlusion of the four major cerebral arteries (Pulsinelli & Brierley, Neurology 28:379, 1978). One day prior to the experiments, both vertebral arteries were cauterized and polyethylene cuffs were placed around both common carotid arteries of all animals. Ischemia was produced by constriction of the carotid cuffs; cuffs on control rats were only manipulated. After 10 or 30 min of ischemia, the carotid cuffs were removed and [1-14C]valine (7.5 µmol/kg, 40 µCi/kg) was injected i.p. at different times during the recirculation period. The rats were killed 70 min after [1-14C]-valine injection, and the trichloroacetic acid-soluble and particulate fractions of 5 brain regions were analyzed. Measurements on control (n=6) and 6-hr post-ischemic (n=6) rats established that: (i) incorporation of [1-14C]-valine into protein was linear for at least 100 min after valine injection, and (ii) although brain valine content was 35-40% higher in the 6-hr post-ischemic animals, the specific activity of brain [1-14C]-valine was similar (within 12%) in both groups. The rates of incorporation of [1-14C]-valine into protein in cerebral cortex, caudate-putamen, hippocampus, midbrain-diencephalons, and brain stem of control animals (n=7) were similar, averaging 32.253 ± 0.003 dpm/mg protein. In rats rendered ischemic for 10 min, protein synthesis in caudate, hippocampus, and cortex was inhibited by 80% during the first hour of ischemia (n=4). The rate of recovery of protein synthesis in the ischemic brain was assessed by measuring rates of control rats were measured at 6 hr of recirculation (n=6), 75-85% at 12 hr (n=5) and 24 hr (n=7), and 100% at 48 hr (n=4). Midbrain-diencephalon, which is partially perfused during the ischemic interval, showed less inhibition of protein synthesis during the first hour of recirculation (65%), and recovery was complete by 12 hr. Protein synthesis in brain stem was unaffected, keeping in the fact that this structure is continuously perfused during 4- vessel occlusion. Following a 10 min ischemic insult, protein synthesis in cortex was 35% of control during the first hour of recirculation (n=5); it recovered to 70-80% at 3 hr (n=6), remained at this level at 6 hr (n=6), and was normal at 24 hr (n=3). Thus, the degree of inhibition of brain protein synthesis and the time to re-establish normal rates of synthesis are dependent upon both the severity and duration of the ischemic insult.


Successful radioautographic visualization of regional brain cerebral blood flow and glucose metabolism has been reported in a variety of species. Gray matter is visualized more readily than white matter in animals studies and human brain. In rats rendered ischemic for 30 min, protein metabolic rate. Recent technological developments in brain scanning have demonstrated that various radiochemical tools can be applied to noninvasive imaging (injected compounds). For 1.5 hr, rats were killed, brains were removed, frozen, and sectioned at 45 min. Cryostat sections are exposed to X-ray film at -80°C. The resulting radioautographs indicate that 135I-Iodobenzene provides an indication of the lipid content of major brain fiber bundles such as corpus callosum, internal capsule, fornix and optic tract. Potential clinical applications of labeled agents that accumulate in brain lipid include the evaluation of the integrity of myelinated CNS pathways.


Portacaval shunting and the associated hyperammonemia increase global cerebral glucose consumption (James et al., 1972), but whether local utilization uniform and to the same degree in animals shunted acutely and chronically is unknown. We used the 14C-2-deoxyglucose (2-DG) method to assess regional cerebral glucose metabolism in rats 1 and 12 weeks after the construction of a PC shunt and in weight-matched controls.

All animals were anesthetized with halothane for insertion of control and experimental catheters and at least 3 hr with the rats awake, normotensive, normoammonemic, and restrained, 0.2 ml/kg of [1-14C]-2DG was injected i.v.; arterial blood was sampled at intervals for plasma glucose measurements. After 65 min, rats were decapitated; the brains were removed, frozen, and sectioned at 20 µm for autoradiography with calcium carbonate plates (standard protocol).

Arterial blood ammonia concentrations were twice the control value of 85±7 µm in rats shunted for 1 week, and were 2.5 times control values in the chronic (12-week) shunted group. On the other hand, averaged 25-35% below the control value of 10.62±0.6 µm in all shunted groups. Local cerebral glucose use (dpm/g/min) for 1 week shunted rats and 8% for 12-week shunted rats. From 2-5 min after injection, a 2-3 fold increase in blood glucose occurred, followed by a 2-3 fold decrease. Blood glucose remained constant through 80 min.


A model of low-dose, insulin-induced hypoglycemia was developed in female rats that produced a constant level of glucose for 1.5 hr. Female rats, weighing 200 g and fasted overnight were anesthetized with ether for cannulation of the tail artery and vein. Rats recovered for 2-3 hr before receiving i.v. glucose-saline. All rats (n=5) became self-supportive and alert, plasma glucose rose from 130 to 250 mg/ml, representing a 2-3 fold increase in blood glucose use; cerebral cortex and white matter were notable exceptions to this trend. Changes in local glucose consumption after normoglycemia thus suggest the differential utilization neurochemical substrates, per se; chronic hyperammonemia and other factors (e.g., the degree of astrocytic pathology, which develops only after 4 weeks) may be contributory.
ELEVATION OF PLASMA TYROSINE LEVELS IN NORMAL HUMANS AFTER ORAL ADMINISTRATION OF L-TYROSINE. Bruce S. Glasser, Eldad Melamed, John W. Growdon, and Richard J. Wurtman. Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

L-Tyrosine, 100 mg/kg or 150 mg/kg was administered to two groups of fasting human subjects. Plasma tyrosine levels were significantly elevated 2-8 hours after tyrosine ingestion. Mean plasma tyrosine levels were maximal at 2 hours, rising from 69 ± 3 to 154 ± 10 nmol/ml after 100 mg/kg and to 203 ± 32 nmol/ml (t ≤ SEM) after the 150 mg/kg dose (p ≤ 0.001 for both groups). The uptake of tyrosine into the brain is dependent on the tyrosine ratio, defined as the ratio of the plasma tyrosine level to the sum of the concentrations of the plasma neutral amino acids (i.e. valine, methionine, leucine, isoleucine, phenylalanine, and typtophan) that compete for the same blood-to-brain transport system. At 2 hours, the mean tyrosine ratio increased from 0.10 ± 0.02 to 0.28 ± 0.04 (t ≤ SEM) after the 100 mg/kg dose and to 0.35 ± 0.05 after the 150 mg/kg dose, indicating that oral tyrosine probably increases brain tyrosine levels in humans. In related studies, the relative immaturity and aberrant development of the central nervous system of maturing experimental animals, not only on gross brain weight and content but also on cell structure and function.

PLASMA TYROSOINE CONCENTRATIONS AFTER TYROSINE ADM.

Tyrosine Plasma Tyrosine Concentration-nmol/ml (t ≤ SEM) Dose 0HR 2HR 4HR 6HR 8HR

100 mg/kg 6.9±3 15.4±10 12.9±7 12.1±11 11.0±10

150 mg/kg 6.9±3 20.3±12 15.8±13 12.9±9 10.6±6

Supported in part by grants from the National Institutes of Health (AM 14278) and the National Aeronautics and Space Administration (NCR-22-009-627)


Undernutrition has been shown to have a profound effect on the central nervous system of maturing experimental animals, not only on gross brain weight and content but also on cell structure and function. In this study, neonatal rats have been exposed to a moderate state of food deprivation to 20 days of age by decreasing the litter size to 1 and removing the dam for 8 hours each day. Neurons of variousReticular Formation nuclei of the brainstem were then examined using modifications of Golgi stains. Dendritic variation has been observed in experimentally with neurons in the form of nodularities and thickenings upon dendrites of lesser thickness and extent, and subtle increases in dendritic spine numbers. The resulting changes suggest that cell development is less advanced. The relative immaturity and aberrant development of these neurons may be the morphologic substrate of behavioral variabilities observed in these animals.
AMINO ACID TRANSPORT INTO RAT BRAIN REGIONS IN VIVO.
Richard A. Hawkins, Anke H Hama, James J. Richter, Barbara L. Halsey and Julieten B. Shockey.* Departments of Psychiatry, Biochemistry and Anatomy, Indiana Univ. School of Medicine, Indianapolis, IN 46222.

The carrier system transporting neutral amino acids from plasma into whole brain has been characterized in normal adult rats (Oldendorf 1971 Am J Physiol.221:1629-1639). The kinetics of this system, for which many neutral amino acids compete, changes considerably in rats with portovascular shunts or during hepatic failure, thus altering the rate of amino acid influx (Ohno et al. 1979 J. Neurochem., in press). The localization of these changes to brain regions, however, has not been determined. In order to measure amino acid influx at the regional level, we developed a technique to measure the influx of amino acids into various regions of the rat brain under physiological conditions, (ie. without disturbing the normal equilibrium of plasma components or altering cerebral blood flow).

A tracer quantity of 14C-labeled amino acid was infused intravenously at a rate which maintained a steady level of label in the blood for 5 to 30 min (Ohno et al., 1978 Am J Physiol. 235:4829-5837). Blood was collected throughout the experiment to obtain the plasma integral of the tracer concentration, and the complete amino acid profile by amino acid analysis. After decapitation, the brain was frozen, sections cut for quantitative autoradiography, and the regional dpm/g obtained by quantitative densitometry. The permeability coefficient (PS) (K x capillary surface area) in different brain regions was calculated from the plasma integral and dpm/g brain after subtracting the background dpm in the area due to contaminating blood (Ohno et al., 1978 Am J Physiol. 235:4829-5837).

The PS values of tyrosine and phenylalanine in four brain regions in normal conscious rats are shown in the following table.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>PS (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>0.066</td>
</tr>
<tr>
<td>Caudate</td>
<td>0.058</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.207</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>0.087</td>
</tr>
</tbody>
</table>

For tyrosine the corresponding influx rates were 6.5, 5.7, 5.5 and 8.8 mmol-ml-1-g-1 in cortex, caudate, hippocampus and inferior colliculus, respectively. Lower substrates with low up-take rates can be studied similarly.

REGIONAL VULNERABILITY OF THE NON-HUMAN PRIMATE BRAIN TO REDUCED BLOOD SUPPLY.
Frank W. Marcoux, Richard B. Morawetz, and James H. Halsey, Jr.* Neurosciences Program, Division of Neurosurgery, and Department of Neurology, University of Alabama in Birmingham, Birmingham, AL 35294.

Twenty-one unanesthetized macaque monkeys underwent temporary middle cerebral artery (MCA) occlusion by a snare technique. Duration of MCA occlusion varied from 15 minutes in some animals to 3 hours in others. Local cerebral blood flow (LCBF) was measured before, during, and after MCA occlusion in cortical and subcortical gray and white matter within MCA distribution. Two to four weeks following temporary arterial occlusion, histologic examination documented cerebral tissue damage and its precise relation to LCFB recording sites. Histopathologic observations at sites having undergone a similar reduction in LCFB for the same duration were compared to define the more vulnerable site. The criteria for increased vulnerability to a decrease in blood flow was the degree of tissue damage. As an example, where 2 or more sites are subjected to a similar degree of ischemia during 1 hour of MCA occlusion, the more vulnerable site is that which suffers the greater histological consequences.

Gray matter sites (insular cortex, putamen, and caudate nucleus) revealed tissue damage of varying degree during 1 to 3 hours of MCA occlusion when LCFB fell to and remained at levels approximately equal to or less than 10 cc/100g/min. Incomplete tissue damage was associated with occlusion durations between 1 and 1.75 hours while total destruction occurred only between 2 and 3 hours of MCA occlusion.

White matter sites (subcortical and capsular areas) appear to be less vulnerable to decreased blood flow than gray matter during 15 minutes to 3 hours of MCA occlusion. No tissue damage was observed at white matter sites until 1.5 hours of MCA occlusion and then only at LCFB values of 6 cc/100g/min or less. Severe tissue damage at white matter sites occurred at LCFB's of 5 cc/100g/min or less after 2 hours of MCA occlusion and generally as a result of a fatal ischemic insult.

Sites in the caudate nucleus demonstrated the greatest vulnerability to reduced blood flow. Alterations in cellularity with apparent loss of neurons alone occurred in the caudate at LCFB levels as high as 10-15 cc/100g/min before 1 and 2 hours of MCA occlusion.

State born to dams fed a low protein diet (RS case) or a normal diet (25% case) during 5 days starting on day 20 postnatal age. Alix and control (C) litter mates of each diet. The results (Table below) indicate that ADX caused significant decreases of 23-40% for whole brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels, and significant increases of 38-17% for tryptophan (TP) levels in both the RS and 25% ADX rats as compared to their respective controls. Plasma constituents showed no significant differences between the ADX and 25% rats of each diet group, except for corticosterone (CORT) levels. The latter findings indicate that the adrenal gland plays a minimal role in producing the changes in plasma albumin (ALB) and fatty acid (NEF) levels and the resulting alteration in TP availability seen in the RS case rats (Miller et al., Exp. Neurol. 57: 130, 1977). However, the effects of long-term protein refeeding on both diet groups indicate that the adrenal gland may regulate brain TP metabolism by: (1) maintaining normal activity of TP hydroxylase, the rate limiting enzyme in 5-HT synthesis, and (2) inhibiting the transport of TP to the brain.

Effects of Adrenalectomy on RS and 25% Cauel-fed Rats

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>RS ADX</th>
<th>25% ADX</th>
<th>25% ADX</th>
</tr>
</thead>
<tbody>
<tr>
<td>(in per group)</td>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT mg/g</td>
<td>6.2 ± 1</td>
<td>3.1 ± 1</td>
<td>3.3 ± 1</td>
</tr>
<tr>
<td>5-HIAA mg/g</td>
<td>683 ± 11</td>
<td>683 ± 11</td>
<td>683 ± 11</td>
</tr>
<tr>
<td>TP mg/g</td>
<td>4619 ± 9</td>
<td>4619 ± 9</td>
<td>4619 ± 9</td>
</tr>
<tr>
<td>Weight kg</td>
<td>1580 ± 3</td>
<td>1580 ± 3</td>
<td>1580 ± 3</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP mg/m</td>
<td>7956 ± 69</td>
<td>8417 ± 309</td>
<td>18570 ± 1605</td>
</tr>
<tr>
<td>5-HT mg/m</td>
<td>354 ± 35</td>
<td>354 ± 35</td>
<td>354 ± 35</td>
</tr>
<tr>
<td>ALB mg/m</td>
<td>76 ± 5</td>
<td>76 ± 5</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>NEF mg/m</td>
<td>37.2 ± 3.7</td>
<td>37.2 ± 3.7</td>
<td>37.2 ± 3.7</td>
</tr>
<tr>
<td>NEF mg/g</td>
<td>1078 ± 10.1</td>
<td>1078 ± 10.1</td>
<td>1078 ± 10.1</td>
</tr>
<tr>
<td>5-HIAA mg/g</td>
<td>75.8 ± 10.2</td>
<td>75.8 ± 10.2</td>
<td>75.8 ± 10.2</td>
</tr>
</tbody>
</table>

Supported by grant HD 06564

Vascular endothelium synthesizes prostacyclin, an unstable prostaglandin (\(t_2\) 2-4 µg, pH 7.4) which strongly inhibits platelet aggregation, increases cAMP synthesis and vasodilates most blood vessels. The capacity for synthesis of prostacyclin from endogenous precursor by rat cerebral arteries and arterioles is 2-4 µg/g tissue/10 min incubation periods at pH 7.4. Highly purified preparations of cerebral capillaries isolated from rat cerebral hemispheres also synthesize prostacyclin in amounts of 12-15 µg/g protein. Topical application of prostacyclin (10-20 µg) for 5 minute periods to the exposed cerebral cortex of rats anesthetized with nembutal caused an immediate striking vasodilation of the surface blood vessels. Local cerebral glucose utilization measured by the 14C-deoxyglucose technique was markedly increased (40-49%) in the parietal and auditory cortex and the effect was dose-dependent. Local cerebral blood flow measured by the 14C-iodoantipyrine method was also significantly increased by 20-25% in the cerebral cortical region of application. Application of prostacyclin to the intact dura dilated the dural blood vessels but had no effect on the glucose utilization of rat cerebral cortex. These studies suggest that prostacyclin released locally from cerebral vascular beds can not only affect blood flow but also increase cerebral glucose metabolism.

Supported by grants from the Medical Research Council of Canada.

BLOOD-BRAIN BARRIER TRANSPORT AND BRAIN SEQUESTRATION OF THE STEROID HORMONES. William M. Partridge, UCLA School of Medicine, Los Angeles, Ca. 90024

Gonadal steroids are concentrated severalfold in brain relative to plasma, but brain corticosterone levels are only 40% of plasma values. A new assumption may exist between the kinetics of brain sequestration of the gonadal steroids vs corticosterone. The rate of brain sequestration of the steroid hormone by brain is a function of the rate of efflux (k2) of the steroid back to blood and the rate of removal (k3) of the steroid hormone by brain. Therefore, by employing an internal 14C reference (R), e.g., butanol or antipyrine, that left the brain as k2, a new k3 can be measured. The rate of change with time in the T/R ratio lead to the estimation of k3 for the steroid hormone. Based on a two-compartment model, the parameters (T/R), k2, and k3 were related as follows:

\[
T/R = \frac{k_2}{k_3} = \frac{1}{k_3} (k_2 + k_3)
\]

Given the k3 values for the reference, the T/R ratios at various times after corticoid injection (0.25-4.0 min) were fitted to the above equation by a non-linear regression analysis. The k2 ± SE:

- Steroid
- Dihydrotestosterone
- Testosterone
- Progesterone
- Corticosterone

- Octanol/Biofilm's

The uncorrelation was found between k3 and the octanol/biofilm's partition coefficient (r=0.18, p < 0.04), suggesting the steroids were not being sequestered by brain lipid. No change in corticosterone k2 was observed in the adrenalectomized rat. Noting the lack of brain retention of 3H-corticosterone was not due to high levels of endogenous hormone. Thin layer chromatography analysis of the radioactivity in the brain after corticoid injection showed progesterone was unmetabolized. Conclusions: The turnover rate of brain binding of blood-borne steroids, that of much higher in the brain for the corticosteroids, a finding which correlates with the much higher volume of distribution of the gonadal steroids in brain relative to corticosterone.


Previous works have shown that changes of carbon dioxide partial pressure increases oxidation of the matrix state levels and reduced nicotinamide adenine dinucleotide (NADH) in neurons of frog dorsal root ganglion. In this work it was expected that this change observed in anaerobic and in aerobic conditions would be blocked differentially with metabolic inhibitors. 2-Deoxy-D-glucose (2-DG) and Amytal were used as inhibitors. The 2-DG blocks the NADH oxidative pathway and Amytal blocks the respiratory chain. Fluorometric determinations of NADH were done on isolated dorsal root ganglion. Fluorometrically reported, pH and pO2 were measured simultaneously. CO2/02 (2.5%/97.5%, 2.5%/97.5%) replaced temporally the O2 and N2 of the atmosphere. All tissues were moistened and records were taken at a temperature of 25°C. NADH showed and immediate and fast decrease (oxidation) after O2 was replaced with CO2/02 and also after N2 was replaced with CO2/02 and then Amytal (5 mM, 5 min). Amytal oxidation of NADH were not observed. After incubation of the preparation in 2-DG (20 min, 30-60 min), oxidation of CO2 mixtures were not observed. After incubation of the preparation in Amytal (5 mM, 5 min), NADH oxidation was observed as CO2 mixtures but NADH did not change. After Amytal was replaced with N2, and after 2-DG replaced N2, Amytal blocked the respiratory chain and reduction of NAD and oxidation of NADH were not observed. The steady state level of NADH is higher than normal. 2-DG blocks the glycolytic pathway (phosphoglucoseo-merase) and NADH is formed less. Under 2-DG inhibition the oxidation of NADH is not observed, neither in O2 nor in N2 with CO2 mixtures, but oxidation of NADH is observed after Amytal (respiration). The Amytal blocks the respiratory chain (NADH-Flavoprotein) and oxidation-reduction was not observed during O2-N2-O2 transitions. Under Amytal NADH and CO2 mixtures can be attributed to lactate formation.

These results indicate that the oxidation observed with the CO2 mixtures comes from the NADH of glycolytic pathway either in aerobic and in anaerobic conditions.

Partially supported by a Grant of Fundacion J.M., Vargas
The metabolic rate for glucose was computed in the cortex and cerebellar nuclei using the quantitative 14C-deoxyglucose (DG) technique. The monkey was operated and conditioned to pull a one pound weight for 12 cm with its right arm while sitting in a primate chair. The animal was made to pull the weight for 10 minutes prior to injection of isotope, and throughout the 45 minute experiment. A bolus of 2DG (100 µci/kg) in 3 ml of saline was injected intravenously. Arterial blood samples (.5 cc) were drawn every 15 seconds for the first minute, at 1 minute intervals for the next nine minutes and at 5 minute intervals for the remainder of the study, in order to determine the arterial plasma 14C and glucose concentrations. Forty-five minutes after 2DG injection the animal was sacrificed with an overdose of Nembutal followed by 10 cc of saturated KC1, the brain was removed and fixed. The brain tissue was cut into 20 microns sections and exposed on x-ray film, Kodak SB5. The tissue 14C concentration was obtained by autoradiographic techniques (.25 mm aperture). The local metabolic rate for glucose was calculated quantitatively from the 14C tissue concentration, the plasma 2DG and the rate and lumped constants as computed by Kennedy, et al (Ann Neurol 4:293-301, 1978). Cerebellar glucose utilization was analyzed from anterior to posterior to lateral and dorsal cortex as well as fastigios, interpositus and dentate nuclei bilaterally. Glucose utilization was more active on the right side. The metabolic rate for labeled DG was from 21.5 to 95.6 in the right cortical areas and 40.7 to 129.4 in the left. It ranged from 16.9 to 55.4 in the left cortical areas and 37.8 to 93.3 in the right. A bilateral difference varied from anterior to posterior according to present concepts of somatotopic localization. Our findings support present information on D-isomer metabolic studies. This study further demonstrates the value of the 2DG technique as an experimental tool in the study of functional neuroanatomical organization.

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The in vivo and in vitro metabolism of L-(3-14C)-hydroxybutyrate in rat brain was studied during development. Rat pups at birth, 6, 15, and 23 days of age were injected with either the D- or the L-isomer of L-(3-14C)-hydroxybutyrate (10 µCi/100 g body wt). After 1 hr the animals were killed and the incorporation into cerebral proteins, lipids, and amino acids was examined. The total incorporation was measured as well as the protein, amino acid, and lipid fractions from either isomer increased from birth thru 15 days of age. Although the pups injected with the L-isomer contained a significantly more protein per gram brain than the controls at ages 6, 15, and 23 days, a greater proportion of the label was found in the non-metabolized L-3-hydroxybutyrate fraction. The labelled brain protein (1.6 X) and amino acids (1.0 X) more effectively than the L-isomer in newborn rats, and was a better predictor of brain protein at 6 and 15 days of age. Both isomers distributed similarly into four brain amino acids at 15 days of age, glutamate, glutamine, aspartate and GABA. Glutamate accounted for 51% of the L-isomer incorporated into brain amino acids for pups injected with either isomer. In newborn rats and at age 6 days greater incorporation into brain lipid was observed with the D-isomer. However at 15 and 23 days of age incorporation from the L-(3-14C)-hydroxybutyrate into brain lipids was 2 X that found for the D-isomer. At 15 days of age 55% of the labelled carbon of brain lipids from animals injected with the L-isomer was found in the cholesterol fraction. At 20 hr 41% of the labelled carbon was found in the sterol fraction when the D-isomer was injected.

The production of CO2 from both isomers was measured in vivo throughout the neonatal period as well as in a series of brain slice experiments from animals at 15 days of age. At 6 and 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. In brain slices from animals 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. In brain slices from animals 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. In brain slices from animals 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. In brain slices from animals in 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. 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In brain slices from animals in 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. In brain slices from animals in 15 days of age the total CO2 released by the oxidation of the D-isomer was 50% of that observed for the D-3-hydroxybutyrate. The presence of the D-isomer is evidenced by the fact that the CO2 production from the D-isomer was significantly higher than the CO2 production from the L-isomer, but was not effective on the oxidation of the L-isomer. As a result, it has been shown that L-3-hydroxybutyrate is incorporated throughout development into brain protein, amino acids and lipids and that its utilization is stimulated by ATP and Coenzyme A.
BRAIN METABOLISM AND NUTRITION


Incomplete ischemia was produced in cat brain by occlusion of the common carotid arteries followed by rapid arterial hemorrhage to a mean arterial pressure of 30-50 torr. After 30 minutes of ischemia, the brain was recirculated for 2 hours, at which time the entire brain was frozen in situ with liquid nitrogen. This freezing technique traps metabolite levels in deep brain regions with negligible ischemic artifact (J Neurochem 31: 299-309, 1978). The frozen brain was sectioned at -15°C at a thickness of 40 µm, and the sections were layered onto an umbelliferone-containing acetate strip. The sections were melted, illuminated with ultraviolet light (366 nm), and the fluorescent images (450 nm) were recorded photographically. Since the fluorescence intensity of umbelliferone is pH-dependent, brain regions with a lower pH would be expected to fluoresce less intensely. Indeed, in the present investigation there were striking regional variations of umbelliferone fluorescence. In brain regions with decreased pH, lactate levels ranged from 20-40 mmol/kg in contrast to the 1-3 mmol/kg present in regions with a normal fluorescence intensity. In addition, these acidic areas of brain showed markedly diminished levels of ATP and phosphocreatine (0.1-0.9 mmol/kg) compared to control levels of 2.4 mmol/kg (ATP) and 4.8 mmol/kg (phosphocreatine).

Thus the use of umbelliferone with frozen tissue sections is a rapid and simple qualitative indicator of tissue pH which may be generally valuable for a variety of applications.

(Supported by PHS grant NS 08803-09)


Paired photic stimuli, separated by varying time intervals, were used to elicit evoked responses as a physiological measurement to study the effect of postnatal undernutrition on the retina of adult rats. Experiments were performed on 12 rats (6 normals and 6 previously undernourished). Normal and undernourished pairs were littermates; the latter were separated from the mother for various intervals each day, leading to undernourishment (50-60% of normal body wt. at 20 days) as we have published previously. After weaning, all rats had free access to lab chow. Electrophysiological experiments were carried out after about two months of nutritional rehabilitation, during which time growth "catch-up" brought the experimental rats up to 85% of control body weight. Animals were anesthetized with pentobarbital (50 mg/kg) and placed in a stereotaxic frame for the recording procedure. The recording electrodes were connected to conventional electrophysiological equipment and were fed into a NIC 1072 minicomputer and simultaneously monitored on a storage oscilloscope. Thirty two evoked responses (1 set) were averaged following photic (2.5 Hz) pair stimuli. Seven sets of pair stimuli (50, 100, 150, 200, 300, 300 and 600 msec time intervals between the pair stimuli) were recorded at 10 min intervals, in both groups of animals. In general the amplitude of the electroretinogram (ERG) in adult, postnatally undernourished rats were reduced by more than 40% (40-60%). In both control and undernourished animals complete recovery of the second response from the pair were observed at 400 msec. In conclusion, postnatal undernutrition had a lasting effect on the ERG without changing the neuronal recovery function, even after prolonged nutritional rehabilitation. (This work was supported in part by Public Health Service Grant NS-13799.)
CEREBELLUM
307 DEVELOPMENT OF CEREBELLAR REGULATION OF FORELIMB TACTILE PLACING MOVEMENTS IN KITTENS. Vahe E. Amassian, Alan Rudell and Larry Eberle* Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, N. Y. 11203.

Contact placing (CP) movements were measured with a TV camera-PDP 11/45 computer system by recording as a function of time the XY coordinates of up to 4 paper spots, which were coated with fluororescein. This first occurred in forelimb and it was recorded with UV light. CP is hypermetric (and slow) in the neonate, the displacements increasing after the first postnatal week before markedly reducing in the 6th-7th week. The trajectory of the forepaw also changes from near 45° to more vertical, the horizontal movement, with the final component directed vertically downwards. The steepening of the initial trajectory results from the forward horizontal vector of movement during elbow flexion helping compensation for the shoulder rotation of flexion of the paw. Thus, with maturation, angular rotations are not only reduced in amplitude but show improved coordination at several joints. ( Flexion only at the elbow would result in an arc-like trajectory.)

Lesions of intermediate and varying amounts of lateral cerebellar cortex or hemiserebellulectomy after the 5th-6th postnatal week result in marked hypermetria (ipsilaterally, with an immaturity (near 45°) trajectory. The hypermetria, but not the immaturity, trajectory changes occurred within 2-3 days of the lesion. By contrast, similar or larger cerebellar lesions made during the 4th and 5th weeks did not increase or trivially increased the pre-existing hypermetria. Thus, the acquisition of neuroanatomical CP was improved, and rotation at a joint reflects the incorporation by the 7th week of cerebellar output into the higher sensorimotor control system.

Treated after the 6th week, cooling or carefully adjusted poliotherapy or lesion of lateral and cerebellar cortex that result in hypermetria do not usually delay the initiation of CP. The average speed of the forepaw often increases during lifting (eg, from 16 to 28 cm/sec), but may remain unchanged. EMGs of biceps and anterior deltoid show an increased activity. The final, landing phase of CP is usually slowed. By contrast, cooling N interpositus reversibly delays, or abolishes CP and activation of a prime mover-biceps. Such findings suggest N interpositus facilitates the primary stimulus in mature CP. An inhibitory output from cerebellar cortex preventing hypermetria.


The influence of parallel fiber volleys set up by a surface (Loc) stimulation on antidromic spike potentials of Purkinje cells (PC) evoked by a brief fastigial (FP) stimulation was studied on the cerebellum of lightly nembutalized albino rats. Data obtained at the level of the PC layer were computed with respect to the time of arrival of the FP volley both superficially and presynaptically in the AC layer. The test/control curve obtained by progressively delaying the interval between stimuli by steps of no more than 200 µsec reveals that the double volley has no significant effect on the FP-evoked PC antidromic spike. The mean latencies to the onset of excitation and inhibition were 55.0 ± 2.4 ms, respectively, were employed to demonstrate the existence of an AC input to the Pf.

310 AUDITORY CORTICAL INPUT TO THE PARAFLOCCULUS: AN ELECTROPHYSIOLOGICAL AND ANATOMICAL STUDY. S. Ausim Azizi, Richard A. Burns and Donald J. Woodward. Dept. of Physiology, Univ. TX Health Sci. Ctr., Dallas, Tx. 75235.

This study was undertaken with the general aim of investigating areas in the rat cerebellum which receive auditory information.

In this report we describe anatomical and electrophysiological evidence that the paraflocculus (Pf) receives information from the auditory (AC) cortex. This finding further supports the hypothesis that the integration of auditory and visual information by the cerebellum is carried out at least by two areas- the well known classical posterior vermal region and the Pf. (Supported by NSF BNS 77-01174 and by the CONICIT Program of Graduate Fellowships)


Extracellular microelectrode recordings were obtained from Purkinje cells in the dorsal rim of the cerebellum of paralyzed frogs during natural stimulation of vestibular and forelimb receptors. In a series of control experiments, recordings were made during natural stimulation of the canals and otolith organs. Stimulation was applied using a specially built turntable which rotated the animal sinusoidally about the longitudinal (roll) axis. Oscillations were performed in the order of 20° ipsilateral side down, or ipsilateral side up, with respect to the recording site.

In the second (or third) set of experiments, the roll oscillations were combined with passive forelimb movement.

In most Purkinje cells, vestibular stimulation by roll (from horizontal plane) evoked simple spike activation in the ipsilateral dorsal quadrant and discharge of a few (1 to 4) climbing fiber evoked spikes upon stimulation in the opposite direction. A selective stimulation of otolith and canal receptors demonstrated that the above responses were due to activation of the two types of receptors. The control experiments were combined with the passive limb movement (test paradigm), the control response showed reduction or absence of the climbing fiber response in Purkinje cells recorded in the medial laterolateral region of the dorsal rim, and little or no change of the simple spike response.

The present results suggest that the climbing fiber input to the vestibulo-cerebellum is involved in error signalling, given that its response only occurs in the absence of the appropriate compensatory limb movement. Thus, when the body-forelimb movement of the paradigm mimicked the compensatory movement of an unauditioned animal, lateral movement, the response was elicited. (Supported by USPHS grant NS-13742 from NICHD and by the CONICIT Program of Graduate Fellowships)
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311 EXCITABILITY CHANGES OF NEURONS IN THE CEREBELLAR NUCLEI FOLLOWING CEREBELLAR SURFACE STIMULATION. Heinrich Bantli, Karl Mayer and Carl J. Hansen, Jr.* Dept. Neurosurg., Univ. Minnesota, Minneapolis, MN 55455

Electrical stimuli applied to the cerebellar surface have been proposed by some investigators as an inhibitory procedure to the Purkinje cells resulting in the inhibition of neuronal activity in the cerebellar nuclei. The present experiments were undertaken to evaluate this hypothesis by recording the activity of neurons in the dentate and interposed nuclei in decerebrate cats before, during and after stimulation of the cerebellar surface. Several of the units were submitted to some tests to assess the level of excitability of neurons before starting the stimulation paradigm. If no decrease in excitability was observed in the PSTH when either the inferior olive, face or forelimb were stimulated, then no records were obtained from the particular cell. If several consecutive cells did not demonstrate any excitability decrease, then the experiment was terminated. The cerebellum was stimulated with capacitively coupled pulses applied with bipolar platinum plates (7.6 mm²) placed on the surface of Crus I and II. The pulse width was 0.1-0.3 msec. and the amplitudes varied from 2-12 ms and the frequency from 10, 50, 100, 150 to 200/sec. The stimulus paradigm consisted of sequential 10 minute recording and stimulation periods for as long as the cell was isolated. Each 10 minute sequence was divided into 10 recording intervals of one minute duration. The neural spike train of each interval was stored as interval histograms on the PDP 11/34. The data was obtained from 63 neurons recorded from 56 cats representing a total of 256 stimulation and 320 recording periods. The analysis included a statistical evaluation of interval histograms and changes in the mean firing rate between stimulation and control sequences. Based on the analysis the following conclusions were possible: 1) Many cells showed significant decreases or decreases in excitability. 2) These excitability changes may be frequency or amplitude dependent for a particular cell in no correlation was observed when the entire cell population was considered. 3) All cells were modulated by the cerebellar surface stimuli as determined by inspection of PSTH although the statistical tests might not have shown the significant changes between stimulation and control sequences. 4) Rebound phenomena were often observed. (Supported by NIH Contract NO1-NS-4-2233.)


It is becoming increasingly evident that norepinephrine may modulate excitation and/or inhibition in the cerebellum. The raphe nuclei (Hoffer et al., 1978). Anatomical studies indicate similarities in the raphe (R) and locus coeruleus (LC) terminations within the various layers of the cerebellar cortex (Hoffer et al., 1978). Furthermore, stimulation of the R complex and LC predominately inhibit spontaneous firing of randomly encountered Purkinje cells. We have compared the effects of raphe and LC stimulation upon spontaneous and evoked climbing and mossy fiber inputs, as well as GABA-mediated inhibition of Purkinje cells.

Whole microelectrodes were used to record single cerebellar cells of Purkinje cells in n-chloroanesthetized, flaxedized, and artificially ventilated cats. Stimulation of the ipsilateral lateral mesencephalic nuclei inhibited the receptor sites elicited mossy and climbing fiber discharges, respectively. "Off beam" inhibition was induced by stimulation of the superficial layers of the cerebellar cortex adjacent to the recording electrode. Evoked simple spike activity decreased after R conditioning from 3.8 to 0.6 spikes/stimulus representing an 81% change (average of 20 Purkinje cells) in comparison to a 62% decrease in spontaneous activity for a similar time period. Reference to evoked activity elicited via sensorimotor cortex stimulation, complex spike activity cell (3.7 to 1.3 spikes/stimulus, representing a 77% change) of 9 Purkinje cells) in comparison to a 67% decrease in spontaneous activity following R stimulation. Conditioning shocks to the R produced a marked augmentation of "off beam" inhibition, indicating that the R inhibits the almost complete disappearance of the endogenous inhibitor of GABA binding.

FOLLOWING CEREBELLAR SURFACE STIMULATION. Heinrich Bantli, G. Di Pino, G. De Montis, and G.A. Genazzani (SPQR, G. Toffano), Institute of Pharmacology, University of Cagliari, Italy.

Kainic acid, handling and haloperidol were used in order to differentiate the specific receptors for GABA and muscimol in the rat cerebellum:

a) kainic acid (4 μg), microinjected into the cerebellar cortex, within 4 days decreased by 70% the number of specific binding sites for muscimol. On the other hand kainic acid markedly increased the receptor affinity for H-GABA; this effect was associated with the almost complete disappearance of the endogenous inhibitor of GABA binding.

b) Differences in the number of specific binding sites for muscimol and GABA were present in the cerebellar cortex, as tested in one monkey (F2). Cooling the inferior raphe was stimulated; whereas, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the postulated enhancing effect of GABA on the inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4).

313 DIFFERENT RECEPTORS FOR GABA AND MUSCIMOL IN THE RAT CEREBELLUM. G. Biggio*, L.G. Costa*, G. De Montis*, and G. Genazzani (SPQR, G. Toffano), Institute of Pharmacology, University of Cagliari, Italy.

Kainic acid, handling and haloperidol were used in order to differentiate the specific receptors for GABA and muscimol in the rat cerebellum:

a) kainic acid (4 μg), microinjected into the cerebellar cortex, within 4 days decreased by 70% the number of specific binding sites for muscimol. On the other hand kainic acid markedly increased the receptor affinity for H-GABA; this effect was associated with the almost complete disappearance of the endogenous inhibitor of GABA binding.

b) Differences in the number of specific binding sites for muscimol and GABA were present in the cerebellar cortical membranes obtained from naive rats and from rats habituated (for 15 days) to the handling maneuvers preceding the sacrifice. Naive rats showed higher binding for H-muscimol but lower binding for H-GABA than habituated rats.

c) The acute administration of haloperidol (4 mg/kg) decreased H-GABA binding but increased that of H-muscimol.

The results suggest that:

a) different receptors for GABA and muscimol are present in the rat cerebellum.

b) Receptors for muscimol are localized on neuronal structures sensitive to kainic acid while GABA receptors are localized on structures resistant to the toxic action of this drug.

c) Stress due to handling causes a sudden change in GABA binding, opposite to that of muscimol.


Flexible cooling probe sheaths were chronically implanted just above the rostral end of the inferior olive (IO) with X-ray assisted stereotactic (4) (2 Pascualian monkeys. Postoperative neurological signs receded in intensity after a few days, and were referable to the tissue damage in the path of the electrode. These were: ataxia of trunk and head (but never the limbs); neck deviation to the implanted side; ipsilateral facial palsy; weakness and loss of both eyes and ipsilateral iris and miosis. Cooling of IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IO for a few min, to a shear temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells. Cooling IOalue of IO-cooling. In the awake condition, the physiological parameters were unimpaired, as might be anticipated from results of partial rostral olive lesions (5). The main finding, below, is more likely to be due to cooling IO rather than, e.g., the nearby rostral regions. GABAergic "off beam" inhibition of Purkinje cells. (Supported in part by NIH Grant HL 7289.)

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3Fellowship in the Muscular Dystrophy Association of Canada
315 THE PARAFLOCCULUS: A POSSIBLE ROLE IN HEAD-EYE ORIENTATION. Richard A. Burns and Donald J. Woodward, Dept. of Cell Bio., Unv. Tx. Health Sci. Ctr., Dallas, Tx., 75235. The paraflocculus is a cerebellar target zone for visual cortical and tectal inputs. As part of the general aim of obtaining the functional significance of the parafloccular visual input, this study was undertaken to further clarify the diverse anatomical connections of the paraflocculus. In this report we describe the anatomical projection (1) the zones of origin of additional afferent inputs to the paraflocculus and (2) the terminal field distribution of the afferent projection from the paraflocculus to the vermis.

Following electropheretic injections (1 µl, 20 min) of a 4% HRP-Tris buffer solution (pH 6.6) into the paraflocculus and reaction with 0.5% methenamine and 0.5% phenyl methyl benzimidazolium labeled afferent neurons were observed 1) bilaterally in the lateral oculus nucleus, prepositus hypoglossal nucleus, locus coeruleus (ipsilaterally preponderant), pontine reticular tegmental nucleus and the basilar pontine gray (contralateral predominance), 2) in the contralateral medial accessory and principal olives, and 3) in the ipsilateral spinal nucleus Y and the cerebellar dentate nucleus. No HRP-positive cells were localized in the vestibular ganglion or nuclei. Of these sources, the largest afferent projection to the paraflocculus originates from the lateral pontine gray; the same pontine region that receives efferents from the visual cortex and superior colliculus.

Injections of H-1-leucine (wells; 0.2-0.5 µl; conc.: 75-100 µCi/µl) into different locations in the inferior olive in the cat were used to demonstrate the topography of olivo-cerebellar climbing fibers. Taking advantage of the complete crossing of this projection bilaterally, it was thus demonstrated in the same animals that complementary injections of the olives correspond to projections to entirely distinct cerebellar regions. This organization of the afferent projections to the cerebellar cortex suggests the possibility that the olives may be organized according to the anatomical cortices thus that there is no convergence to any cerebellar cortical region from different olivary origins. On the other hand, small localized injections of the olives demonstrate extensive projection areas distributed at right angles to the longitudinal axes of the olives. This indicates a great deal of divergence in the olivo-cerebellar projection. A general organization of the olivo-cerebellar projection was recognized. The caudal third of the olives projects to the intermediolateral and lateral parts of the first four lobules and to the paramedian lobule. The intermediate and lateral parts of the lobules V and VI and most of the crura are projected upon the rostral two thirds of the olives. Lateralmost portion of the crura receive projections from the rostral third, only. More specifically, the caudal medial accessory olive (MAO) projects to a sagittal strip of cortex located next to the midline, in all ten lobules. In the anterior lobe, from medial to lateral, for additional sagittal projections: 1) the dorsal accessory olive (DAO); 2) the MAO; 3) the DAO again and 4) the principal olive (PO). In the posterior lobe lobules VIII and IX, additional sagittal projections from the DAO and rostral MAO were seen next to the midline MAO distribution. No clear data were obtained for lobule VII. In the paramedian lobule, there are three sagittally arranged groups of projection. From medial to lateral, they correspond to the DAO, MAO and PO. The crura receive projections from the MAO and DAO medially and from the PO, laterally. The paraflocculus receives its projections from the MAO and PO. (Supported by a Grant from the Canadian MRC to the Group in Neurological Sciences, University of Montreal)
ALTERATIONS IN CORTICAL LAYERING SURROUNDING CEREBELLAR FISSURA PRIMA

The three-layered appearance of the adult rat cerebellar cortex as it surrounds each lobule is well documented from spinal material to arboreal layer as pial cortex, molecular layer and Purkinje cell layer and internal granular layer. However, we have regularly observed alterations in this three-layered arrangement of cortical surrounding regions in addition as well as in neonatal rats where the external granular layer is still present, adding a fourth layer. In 80 of 100 rat brains analyzed, the cortical layers surrounding fissura primae were found to be altered from the four (neonatal) or three-layered (adult) arrangement found in the majority of the remainder of cerebellar cortex. This common alteration extends long distances on either side of the midline causing fissura primae to become more and more shallow from its lateral to medial extent. In sections sectioning the lateral edge of the alteration process on the neonatal cerebellum, the external granular layer and pial cells disappear leaving an expanse of molecular layer lying between internal granular layer cells of lobules V and VI. Proceeding medially toward the mid sagittal section, the cells of the internal granular layer of lobules V and VI are situated closer together than usual, and there are regular alternating layers of the same kinds of cells as are usually present in a monolayer, but are displaced: sometimes clusters of these cells are found within the outer cortico-vestibular cortex. With more complex situations, tissue layers of molecular, granular, and Purkinje cell groups surrounding fissura primae were common places in regions of cerebellum as far lateral as the 320 microns from the midsagittal, and still more dorsolateral extent of such alterations measured was 400 microns. Although this work was an analysis of cerebella from unstressed control animals, we have regularly observed altered layering patterns in cerebella from animals with induced environmental stresses applied during cerebellar development. Therefore, analyses of staggerer corticocerebellar cortex, resulting from applied stresses should take into account the normally occurring alteration in layering patterns reported here. This work was supported by NIH AI 14665, NFSMRS 77-00174.

GRANULE CELL DEGENERATION IN THE CEREBELLUM OF THE PURKINJE CELL. Dean E. Hillman and Suzanne Chen. Dept. of Pathology and Immunology, Washington University School of Medicine, Indianapolis, IN. 46223, USA.

The pcd mutation in mice is autosomal recessive. Homozygotes will die first shows severe defects. Newborn PCDs are unable to compensate significantly by increasing their own numbers. The results will be discussed in terms of the action of the sg gene product. Supported by Nat. Fdn-March of Dimes, Basil O'Connor Grant and NIH Grant PHR 12213-01.

DETERMINATION OF POSTSYNAPTIC MEMBRANE STRUCTURE IS INTRINSIC TO THE PURKINJE CELL. Dean E. Hillman and Suzanne Chen. Dept. of Pathology and Immunology, Washington University School of Medicine, Indianapolis, IN. 46223, USA.

A fundamental question in understanding the development of neuro-architectural differentiation is whether the extent of postsynaptic specialization is determined by affinity or intrinsic factors. The postsynaptic specialization is determined by afferent connections or intrinsic factors. A reduction in the number of synaptic contacts on each Purkinje cell results in a 25% control diet was found to be reduced in number, and there is a 300,000, 1966). Here we show that in the malnourished female offspring, the number of synaptic contacts on each Purkinje cell is also reduced; however, the total synaptic contact area remains constant for each cell. An 8% protein diet with calories equal to 0% of control diet was found to be reduced in number, the offspring had a 40% total reduction in number of granule cells in excess of 26% while granule cells of males were decreased by only 5% of the control. At the same time, total Purkinje cell numbers remained unchanged in both sexes. Quantitation of molecular layer volume and synaptic density indicated that total number of spine synapses in cerebellum of males was unchanged while in the female it was reduced as much as 10%. Thus, the male Purkinje cell-parallell fiber synapses were completely compensated by an increase in postsynaptic sites on parallel fibers of granule cells. The females also compensated but had a residual deficit of about 10% fewer spine synapses on controls or malnourished males. Severely affected females displayed giant spines and attained a synaptic deficit greater than 30%. Electron microscopic quantitation of the spine-synaptic specialization contact length of 8% of the experimental males, and experimental females with and without giant spines showed that the area of postsynaptic thickening on spine processes increased inversely with the number of Purkinje cell-parallell fiber synapses. The average synaptic contact area in males was unchanged while the females had an area increase of 20%. In severely affected females which displayed giant spines, this average area was over 30%. We conclude that (1) the total postsynaptic specialization area on each Purkinje cell remains constant following a 25% diet and (2) the male Purkinje cell-parallell fiber synapses are not postsynaptic specialization-macromolecules distribute to available parallel fiber synaptic sites, and (3) Purkinje cell spines can synaptically compensate for a number of postsynaptic specializations. The constancy of total spine postsynaptic area on each Purkinje cell indicates that postsynaptic junctional macromolecules must be produced in the Purkinje cell and distributed through the genome, and thus serve as one of the parameters that establish fundamental neural organization in the cerebellum. (Supported by USPHS grant HD-10934 from NICHHD.)
was found to give rise to a sparse contralateral projection, afferent centres within each of the subdivisions of LRN. 

Extensive integration of converging impulses from two or more main portions. Within the LRN was demonstrated with the method of Fink and Heimer. An extensive input from the spinal cord was found to terminate predominantly on the ipsilateral side throughout the entire rostro-caudal extent of the LRN, except for a small rostro-caudal medial area in the magnocellular portion. The cortical projection terminated diffusely within the rostral aspect of the contralateral magnocellular part of the nucleus. The fastigial nucleus terminates predominantly on the ipsilateral side throughout the entire rostro-caudal extent of the LRN, except for a small rostro-caudal portion of the nucleus, with less to the parvocellular and subtrigeminal portion. 

The LRN therefore receives spinal and supraspinal projections that terminate within specific areas of the nucleus in a partially overlapping fashion. This pattern of input allows for an extensive integration of converging impulses from two or more afferent centres within each of the subdivisions of LRN.

(Supported by the Medical Research Council of Canada.)

**WIDE**

**SUPERIOR COLLICULUS PROJECTIONS TO TACTILE AREAS OF RAT CEREBELLUM HEMISPHERES. Jeffrey J. Kassel, Dept. of Neurophysiology, Univ. Wisconsin, Madison, WI 53706.

The spatial organization of the projections of the superior colliculus in the contralateral cerebellar hemisphere and posterior vermis was examined using micromapping techniques in barbiturate-anesthetized rats. Using two independent, ball-tip, tungsten microelectrodes, multiple unit clusters were recorded from tactile areas in the stratum griseum intermediate of the superior colliculus (SC) and the granule cell (GC) layer of cerebellar cortex. The receptive fields of both areas were defined by threshold mechanical stimulation of cutaneous tissues. Electrical stimulation (monopolar, biphasic pulses; 10-40 µA; 3/sec) through the SC electrode evoked responses in limited regions of the contralateral GC layer of cerebellar cortex. In most experiments, the SC stimulating electrode remained stationary while the cerebellar electrode was used to map the region of the GC layer activated by SC stimulation. In a few experiments, the cerebellar recording electrode remained fixed while the SC electrode mapped the area of effective stimulation. Puncture densities up to 75/mm² were necessary to fully determine the spatial pattern of the SC projections.

Major results are: (1) Cerebellar responses to SC stimulation are found in the GC layer of contralateral Crus I and II and paramedian lobules. (2) Peripheral receptive fields of the interconnected SC and GC loci include primarily facial structures (especially vibrissae, crown and eyelid). (3) SC sources project only within the confines of GC patches (defined by Shams et al., BBE 15: 94-140, 1978) with homologous receptive fields. (4) Latencies of GC layer activation following SC stimulation are typically 3.0 - 3.5 msec. (Supported by NINCDS fellowship F32 NS0047 and NSF grant BNS 77-16230.)

**WITHDRAWN BY AUTHOR**

In addition to the widespread deficit of myelin in quaking mice, axons in the cerebellar white matter exhibit focal swellings up to 10 μm in diameter (Suzuki & Zagoner). Here we report abnormalities in the ultrastructure of synaptic terminals in the cerebellar nuclei of 3-6-14 old quaking mice. Synaptic terminals in control mice resemble those in the rat (Chan-Palay). Synapses in quaking mice share essential features with those of the control mice; however, many boutons showed dystrophic alterations --- including enlargement of the terminals; increased content of mitrochondria, also often enlarged; membrane whorls; and excesses of synaptic vesicles. In addition to the apparently normal synaptic vesicles, many endings contain dense clumps of very small (150-250 Å) vesicles which may be abnormal synaptic vesicles. These are seen both in profiles which are otherwise unremarkable and in profiles with the dystrophic changes described above. The affected boutons maintain their synaptic adhesions with the normal range of post-synaptic sites --- i.e., somata and dendrites.

To assess the possible loss of synapses, we measured the fraction of surface of neuronal somata in the medial cerebellar nucleus apposed to synaptic boutons; this was not reduced in quaking mice. Thus, net loss of synapses does not occur and degeneration of terminals (if it occurs at all) is followed by replacement. We believe that the majority and perhaps all of the dystrophic terminals and those with vesicle clumps belong to Purkinje cells. Many altered profiles closely resemble Purkinje cell boutons of normal mice (moderately dense ground substance, characteristic cisternae of ER, and characteristic vesicle shapes). In addition we find the vesicle clumps and other changes in the superior and lateral vestibular nuclei and in the infra-ganglionic plexus of the cerebellar cortex, where Purkinje cell axons are known to terminate. Finally, mossy and climbing fibers in the cerebellar cortex were not seen to be affected.

The relationship between the deficit of myelin and the axonal and synaptic abnormalities is most; however, the behavioral symptoms of quaking mice may derive, at least in part, from abnormalities within neurons rather than from defective myelination.

The clumps of small vesicles found in the mutant are to our knowledge unique and may signal an altered life cycle of synapses.

Supported by NIH Grant NS-09994.

GLIAL CELL ANTIGENS DETECTED BY MONOCLONAL ANTIBODIES. Carl Lagenaur*, Ilse Sommer* and Melitta Schachner. Institute of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 347, 6900 Heidelberg, 3.2.2.

Monoclonal antibodies that distinguish glial subpopulations in mouse cerebellum were detected in frozen sections by indirect immunohistology. Mice immunized with crude mouse cerebellar membrane preparato or with bovine corpus callosum provided spleenocytes that were fusing with NS 1 mouse myeloma by the technique of Lemke, et al. (Nature 271:249, 1977) to yield antibody producing hybridomas. Antibody M-1, arising from crude cerebellar membrane immunizations, stained fibrous astrocytes in the white matter, but did not stain other cerebellar glial cell types. Although Bergmann glial cells in wild type cerebella were M-1 negative, this cell type was M-1 positive in homozygous weaver mutant cerebella.

Antibody C-1, arising from bovine corpus callosum immunizations stained only Bergmann glial cells in adult mouse cerebellum. C-1 was detectable in Bergmann glia at least as early as postnatal day 2 when glial fibrillary acidic protein is not yet detectable by immunofluorescence. Astrocytes in presumptive white matter were C-1 positive in young mice but became C-1 negative in adults. These antibodies provide sensitive probes for glial development and cell separation.
CEREBELLUM

MOTOR CORTICAL MODULATION OF CEREBELLAR AND RUBRAL OUTPUT.
Kenneth D. Laxer* and Mark J. Hallett*.
The Rockefeller University.

The purpose of these experiments was to determine the manner in which the motor cortex regulates cerebellar and rubral output. An array of microelectrodes was implanted in the motor cortex, and action potentials were recorded from each electrode. The stimuli were delivered through each electrode to evoke movements, and then the movements were recorded with respect to the cortical site from which they were evoked. The movements were suppressed by stimulation of a cortical site which evoked movement at an adjacent joint or at the same joint in a different plane. Red nucleus neurons had the same response patterns as cerebellar neurons although a short latency facilitation, suppression, and a second latency suppression were observed. The second latency suppression was either preceding or following the response presumably mediated by the cerebellum. Not all components of the response (i.e., the corticorubral facilitation and the cerebellar-mediated suppression) were present in every cell, but in cat cortex a pattern was present in 22% of 40 neurons which were tested, and 7 of 20 responded with a facilitation after a second latency suppression. These patterns are similar to those found in cats with an input from the precentral cortex, whereas 17 responded with pattern A, and 3 with pattern B. In summary, the predominant patterns found in the cerebellar nuclei and the red nucleus neurons were that neurons driven by passive movement of a joint in one direction were suppressed by stimulation of the cortical site from which movement was evoked in the opposite direction.

SEMOTOTOPIC ORGANIZATION OF CLIMBING FIBER PROJECTIONS FROM CORTICUS DIFFERENTIATES TO LOBULE V VERMAL CORTEX OF THE CAT. CEREBELLUM.
Kenneth D. Laxer*, Lee T. Robertson, and Ann Mason*.

Previously we have demonstrated that climbing fiber projections to the cat cerebral cortex show no distinct topographic distribution of cutaneous components. Although small cutaneous receptive fields were identified, the cortical map had a similar distribution of receptive fields to those seen in the somatosensory cortex. These projections formed a complex medial lateral organization of patches that were elongated in the anteroposterior dimension. These patches covered the entire surface of the cerebral cortex, but the patches were more prominent in the lateral, anterior quadrant of the body; the face was represented lateral and the distal forelimb mediolaterally. The present study investigated the somatomotor climbing fiber projections from the vermis of the lobule V in the cat cerebellum. Extracellular single unit Purkinje cell responses to natural stimulation were recorded in both normal animals and cats with thalamotomy. A computer-controlled punctate stimulus was used to delineate those areas which could activate climbing fiber responses (CFR). Peristimulus histograms were computed for CFR's, and an abrupt D.C. depolarization caused a large conductance change which subsided and then neurons in the intermediate grey, N.Y., N.Y., 10021.


A previous study demonstrated that inferior olive neurons in the cat cerebellum could be modulated by electrical stimulation in vitro to produce complex and long term excitability changes. These changes are presumably mediated by the direct corticorubral projection, which has a different function from that produced by electrical stimulation of the cerebellar cortex. The present study used a different method to produce long term excitability changes in inferior olive neurons. In addition, bath application of harmaline (known to produce a specific receptor-mediated excitation of inferior olive neurons) increased the number of calcium spikes in inferior olive neurons. These findings suggest that the inferior olive is capable of complex and long term excitability changes. These changes are presumably mediated by the direct corticorubral projection, which has a different function from that produced by electrical stimulation of the cerebellar cortex.

Trained saccadic eye movements and fixations. Successful completion of a sequence of fixations and saccades was dictated by training the animal to look at sequential light emitting diodes. These were mounted in a square matrix (10 x 10) placed at 5 degree intervals in horizontal and vertical orientations on a tangent stimulus board. Chronic unit recording was carried out, while the horizontal and vertical components of the eye movements were calculated from optical densities x volume, measured planimetrically, and expressed below as percent of total (terminology of Faull, JCN 178: 495, '78).


Kainic acid (KA). This structural analogue of glutamic acid, has been proposed as a specific lesioning agent which destroys cell bodies possessing glutamate receptors while sparing fibers of passage and neurons without glutamatergic input. However, a few studies have suggested widespread and/or non-specific effects of intracerebral KA injections. In the cerebellum, glutamate is the putative neurotransmitter of granule cells while the other identifiable cell populations (Purkinje, basket, stellate and Golgi II cells), which receive afferent input from granule cells, are not glutamatergic. This cytoarchitecture provides a model system which can be used to assess the mechanism of KA neurotoxicity. A previous report concerning injection of this agent into the rat cerebellum indicated a lack of KA action consistent with a neurotoxic effect mediated via glutamate receptors. The present study was undertaken to further delineate the mechanism of KA toxicity in the mouse cerebellum and to evaluate the resulting reaction to injury in this species.

Several doses of KA (between 0.4 µg and 1.0 µg) in 0.4 µl saline were injected into the cerebellum of weanling mice. Two or 7 days after injection the mice were perfused with 1% glutaraldehyde and 0.5% paraformaldehyde in 0.12 M phosphate buffer. Sagittal sections were fixed in osmium tetroxide, dehydrated, and embedded in Epon-araldite for light and electron microscopy. In contrast to previous studies, substantial numbers of granule cells throughout most of the affected cortex were damaged by KA. This result suggests mechanisms of toxicity other than those proposed for cells with glutamate receptor sites. Of those cells receiving glutamergic input, basket and stellate cells were severely affected while Purkinje cells were often spared. The deep cerebellar nuclei, which receive no identified glutamatergic innervation, also showed some neuronal destruction. Additional pathological changes included macrophage infiltration in all cortical layers and herniations at sites remote from the needle track. Thus, in the mouse cerebellum unexplained factors other than glutamatergic innervation apparently are responsible for the major neurotoxic effects of KA.

This research was supported by the NIH Biomedical Research Support Grant of the College of Osteopathic Medicine, Michigan State University.


Experiments were performed on decerebrate cats which were either unanaesthetized or anaesthetized with halothane. The tendon of the gastrocnemius muscle was mounted on a muscle puller, and a fine wire was inserted into the belly of the muscle to record multiple unit EMG activity. Responses to muscle stretch were recorded in the presence and absence of dentate stimulation at frequencies ranging from 5-100 Hz. To ensure that the activation of rubrospinal and reticulospinal pathways was not accompanied by the activation of spinocerebellar fibers, the responses evoked in the cervical spinal cord by the dentate stimulus were monitored. The electrode was positioned so that only descending projections were activated. When these conditions were satisfied, dentate stimulation produced one or both of the following changes in reflex activity: (a) depression or total elimination of the response of one or more motor units; (b) an increase in the phase lead of the response of individual motor units. These effects were pronounced and occurred more frequently among motor units producing large spikes than those producing small spikes. In addition, when several motor units were affected, those with the largest spikes were always affected first after the onset of dentate stimulation. This observation was made at several recording sites within the same muscle. These data indicate that descending projections from the brain stem which are activated by the dentate nucleus produce characteristic changes in the excitability of stretch reflexes and suggest the possibility that motor units are differentially affected by these descending systems according to their size. (Supported by NIH Grant # R01-NS-09447 and NIH contract NS 4232).

Supported by Grant PHR-1048.
AN ELECTRON MICROSCOPIC AND HRP STUDY PROVIDING EVIDENCE THAT BOTH THE CORTICOPONTINE AND CEREBELLOPONTINE SYSTEMS ARE EACH COMPOSED OF TWO SEPARATE NEURAL POPULATIONS. Gregory A. Mihailoff and Carl R. Watt*, Dept. Cell Biology, Univ. Texas Health Science Center at Dallas, Dallas, Tx.

A part of ongoing studies concerned the synaptic organization of the rat basilar pontine nuclei (BNP), an attempt has been made to 1) identify axonal boutons of the corticopontine and cerebellopontine systems using routine degeneration techniques and 2) to demonstrate the cells of origin of these two systems by injecting horseradish peroxidase (HRP) into the BNP. Our results indicate that following degeneration, two separate populations of boutons in the BNP appeared to undergo degeneration. Most numerous were small boutons (less than 1 μm) exhibiting the typical degeneration and observed to contain small dendrites and spines. Also apparent (but in smaller numbers) were large boutons (1-3 μm) terminating on neuronal perikarya of pontine neurons exhibiting a filamen­

tous reaction prior to becoming electron dense. When lesions were restricted to sensorimotor cortex, the same mixture of dark and filamentous boutons were noted, however, when lesions involved visual cortex, essentially only filamentous boutons were observed. Similar results were obtained when the brachium conjunctivum was used generally. Demonstration of the presence of these axons projecting to the BNP. Numerous large boutons contacting a characteristic cluster of dendritic protrusions underwent filamentous degeneration while at the same time a population of small boutons having a different postsynaptic locus exhibited electron dense degeneration. Taken together, these results suggest that both the corticopontine and cerebellopontine systems might each be composed of two neuronal populations, one type providing fila­

mentous degenerating boutons, the other dark degenerating boutons. To test this notion, HRP was injected into the BNP using a central approach. Labelled neurons within sensorimotor cortex were distrib­

uted throughout layer Vb while in the cerebellar nuclei, numerous large spherical neurons were labelled with a lesser number of smaller spindle-shaped somata. These findings, when correlated with the degeneration studies suggest that the sensorimotor cortical input to the BNP consists of 1) corticospinal axon collaterals (dark boutons, somata in deep layer Vb) and 2) corticobulbar or direc­

torticopontional axonal terminals (filamentous boutons, somata in superficial layer Vb). Similarly, the cerebellopontine system con­

sists of two components, large neurons which provide collaterals (filamentous boutons) to the BNP as they project rostrally to the red nucleus and thalamus and colliculi (dark boutons) of smaller somata which project caudally.

Supported by NSF grant BNS 77-03265 and NIH grant NS 12644.

CEREBELLUM


The rat cerebellum contains both 8-1 and 8-2 adrenergic receptors. Although 8-1 adrenergic receptors comprise 18% of the total number of receptors in the cerebellum at 2 weeks old, 8-2 adrenergic receptors comprise only 2% of total 8-adrenergic receptors in the cerebellum. Since we have previously shown that in the rat cerebellum, 8-1 adrenergic receptors exhibit a unique rate of degradation, it was of interest to examine the cellular localization of the 8-1 and 8-2 adrenergic receptors in the cerebellum.

Rats were subjected to X-irradiation on days 1, 6, 8, 10, 12 and 14 of life. Conventional histological techniques were used to confirm the nearly total absence of the late-maturing granule, basket and stellate cells. The early-maturing Purkinje cells were spared, however, and both histological and electrophysiological studies suggest that these cells are relatively "normal" after X-irradiation.

Rats were killed at 2 or 6 weeks of life and 8-1 and 8-2 adrenergic receptors were measured in homogenates of the cerebellum and cerebral cortex. The density and total number of each receptor subtype was compared to that in control animals subjected to sham X-irradiation. No differences were observed in the concentration of 8-receptor subtypes in the cerebellar cortex of X-irradiated animals as compared to controls. Large changes were seen, however, in the cerebella of X-irradiated animals. At both ages a large (60-75%) decrease in the weight of the cerebellum was observed. There was, however, a 62-70% increase in the density of 8-adrenergic receptors in the X-irradiated cerebellum. The specific activity of 8-2 adrenergic receptors in the cerebellar cortex of X-irradiated animals was unchanged at 2 weeks of age, but decreased by 50% at 6 weeks of age. Similar results were obtained when the brachium conjunctivum was X-irradiated. This indicates that the cellular components destroyed by X-irradiation are relatively enriched with respect to 8-adrenergic receptors. Conversely, the specific activity of 8-2 adrenergic receptors was unchanged at 2 weeks of age, but decreased by 50% at 6 weeks of age. Similar results were obtained when the cerebellum at six weeks of age showed that the number of 8-2 adrenergic receptors per cerebellum had decreased by 8% in the X-irradiated animals, but that the number of 8-1 adrenergic receptors per cerebellum had decreased by only 33%. These data are consistent with the hypothesis that there are 8-1 adrenergic receptors on Purkinje cells of rat cerebellum. (USPHS NS 13289 and 019199).


When a monkey moved its eyes, pursuing a sinusoidally oscillating visual target, simple spike discharges of Purkinje cells of the flocculus showed a cyclic modulation. This modulation frequently occurred in phase with the eye velocity curve, exhibiting a ½ π radians phase shift from the horizontal EOG. However, the activity in these cells did not occur in phase with the maximum eye velocity. A wide range of phase shifts was observed between the activity and the EOG. The activity in these cells was not a velocity component in addition to the eye velocity component. The velocity component could be demonstrated by changing the frequency of oscillations of the visual target. The eye velocity component included a non-velocity component. The peak firing rates were found to increase linearly with higher frequencies which were associated with higher peak velocities in Purkinje cells showing a phase shift from the eye velocity curve, a source of the non-velocity component was the activity representing eye position. The eye position component was demonstrated in the following ways: (1) These cells exhibited tonic activity which was proportional to eye position during steady fixation of a stationary target. (2) During a zig-zag eye movement, tracking ramp target movements of a constant velocity, the firing rate of these cells increased or decreased depending on the direction of the eye movement. (3) When sinusoidal eye movements were executed, pursuing a target oscillating in the central, right, or left visual fields, the level of the cyclic modulation in activity varied markedly depending on the field of eye movement. (4) Eye velocity and position components of unit activity were dissociated during eye movements involved with pursuing a complex target motion driven by a composite of sinusoids of different frequencies. In these conditions, the level of cyclic modulation in eye movements with the pursuit eye velocity as well as the peak eye position were different in every cycle and the potential predictability of a pure sine wave was eliminated. The peak rates during eye movements were continuously correlated with the eye position curve (EOG) or eye velocity curve (the derivative of the EOG). The activity during pursuit eye movements was correlated with the eye velocity and eye position signals, although their proportions differed from unit to unit. (Supported by NIH Grant ET 01501).

CEREBELLUM


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Molecular weights of approximately 200, 145, 120, 85, and 65, 50 and 30x10^3 daltons (P200, P145, ...) as estimated by one-dimensional finger print mapping of peptides by SDS-polyacrylamide gel electrophoresis in gradients from Staphylococcus aureus V8 protease digests. The two major iodinated proteins are the glycoproteins solubilized plasma membranes of embryonic cerebral, and other brainstem nuclei, such as the vestibular nuclei, labelled bilaterally.

Afferents to the nodulus were shown to arise mainly from the dorsal cap of Kooy (dc), the ventrolateral outgrowth of the dc (vlo) and some cells in the rostral part of the medial accessory olivary (MAO). Labelled cells consistently appeared in dc and vlo only when the spread of HRP involved the nodulus. These findings are similar to those cell patterns reported in the rabbit (Alley, et al., Brain Res. 98:589-598; Hodvik and Brodal, J. Comp. Neurol. 176:269-280, 1979). In injections involving mainly the uvula without apparent nodular spread, heavy cell labelling was found throughout nucleus B and the dorsal medial cell column (dmc) and in the rostral parts of the MAO, as has been previously reported in cats (Brodal, J. Comp. Neurol. 166:417-426, 1976). In some nodular injection cases varying amounts of leakage occurred in passage through the folia of the uvula and in all available cases the HRP spreads into the overlying ventral uvula, part of which belongs to the vestibulo-cerebellar pathway. Current experiments suggest that the possible B and dmc cell projections onto the nodulus, but further small injection experiments utilizing various angular approaches to the nodulus are needed to settle the question. These experiments, however, by available experiments are too large to confirm the topographical projections of B and rostral MAO onto the nodulus as suggested by studies in the rabbit (Greenewegen, Voogd and Freedman, J. Comp. Neurol. 183:551-602, 1979).

Anterior lobe injections using this horizontal approach into the uvula and some parts of rostral cerebellum are located in the caudal ventralolateral MAO, the caudal ventralolateral DMO and B, results similar to those reported by Brodal and Walberg (J. Comp. Neurol. 172:85-104, 1976) using a more dorsal approach.

Patterns of labelled cells projecting from the vestibular nuclei and other brainstem nuclei to the folia composing the vestibulocerebellum will be discussed.

Supported by: NARA Task 970-05-02-07.
ERRONEOUS ZONES OF THE CEREBELLAR FLOCCULUS.

The climbing fiber (CF) projection from the inferior olive to the cerebellar cortex is organized into a number of zones oriented normal to the long axis of the folia. By taking an eclectic approach focused on the folliculus, a proposal on the nature of CF zones may be offered in specific behavioral terms. Groening and Voogd (1971) have demonstrated in the monkey that the CF projection from the dorsal cap of the inferior olive to the rostral folliculus delineates three zones — a central zone from which neurons receive the CF signal and two bordering zones which receive from the rostral dorsal cap. In this laboratory we have shown that dorsal cap CFs in rabbit are visually responsive in a direction and speed selective manner. The CF signals constitute error signals (retinal image slip) indicating deviation from optimal motor performance. In addition, we have also shown that three preferred directions in visual space are defined by the visual CFs. Two of the preferred directions (horizontal, up with a posterior component) are signaled in the folliculus from the central zone while the third direction (down with a posterior component) is signaled from the contralateral eye. Recordings from the dorsal cap have shown that the horizontal direction is represented in the caudal part of the dorsal cap while the off-vertical directions are represented more rostrally. Taken together, the anatomy and physiology indicate that the central zone of the folliculus is related to horizontal eye movements while the two bordering zones are related to off-vertical eye movements. These predictions are borne out by the finding (Ito, et al., 1974) that visual stimulation with a rabit folliculus three zones can be distinguished on the basis of the directions of the evoked eye movements. Horizontal movements are evoked from a central zone while off-vertical movements are evoked from the bordering zones. The three directions of the evoked eye movements have an internally consistent relation to the three zones established from the visual CFs. Thus, each of the three visual CF zones in the folliculus is associated with one of these specific directions, which may be viewed as the 'motor' or compensatory eye movements. For the folliculus, the coordinates represented by the CF zones can be readily referred to the extrinsic world geometry. Just as cerebellar mossy fiber responses interpretation of CF zones in terms of coordinates of sensory-motor space will not likely be so directly referable to the external space. Supported by USPHS Grant NS-13742 from NICHD.

An in vitro slice preparation of guinea pig cerebellum has allowed investigation of the voltage-dependent electroresponsiveness of Purkinje cells at somatic and at different dendritic levels. While it was previously reported that, in these neurons the somatic action currents are mainly carried by sodium, and the slower dendritic action potentials by calcium current (Llinas & Sugimori, Soc. Neurosci. Abst., 4, 1978), we have recently identified a sodium-dependent spike was observed. Thus, following blockage of the slow calcium current (by adding either manganese, cobalt or calcium salts to the superfusing fluid) or by simply changing calcium with magnesium), direct stimulation of the somata generated, besides simple spikes, a slowly rising and prolonged all-or-none response. This response filtered by the low threshold in the plateau phase of the fast spikes. This plateau was maintained as long as the depolarizing current was applied and its duration outlasted the stimulus. The duration of the plateau after termination of the stimulus may range from a few milliseconds to several hundred milliseconds, depending on the stimulus amplitude. A test pulse applied during the plateau indicated a fourfold increase in membrane conductance during this period.

Application of 10^{-5} tetrodotoxin to the bath reversibly blocked both the action currents and potentials on the plateau response. Similar findings could be obtained if sodium was replaced in the superfusion fluid by tris or by choline chloride. The plateau level of this all-or-none response was again the velocity or the direction of movement which were associated with the test spot movement. The encoding within the cerebellar cortical neurons during highly controlled finger pressures was therefore that of the test spot movement while the two bordering zones are related to off-vertical eye movements. Further, the discharge pattern of Purkinje cells indicates that the majority of these neurons are inhibited during the co-contraction of antagonistic muscles.

ACTIVITY OF NEURONS IN THE CEREBELLAR CORTEX RELATED TO PARAMETERS OF A CONTROLLED ISOMETRIC PREHENSION TASK PERFORMED BY MONKEYS ALLAN M. SMITH and DANIEL BOURBONNAIS* (SPON: H. H. JASPER). Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec.

Recent studies of the cerebellum have emphasized the role of this structure in the control of voluntary movements. In the present study was designed to examine the discharge of cerebellar cortical neurons during highly controlled finger forces. The monkeys were trained to perform isometric forces. Prior to the hand held strain gauge for force juice reward. An exhaustive analysis of 26 zone and intrinsic muscles of the hand indicated that the task is accomplished by co-contraction of antagonistic muscles. To date recordings have been made from 136 neurons in the paramedian border area between the anterior and posterior lobes (lobules V and VI). Sixty-six units had receptive fields on the forearm, wrist and fingers and discharged in relation to the task. In general, neurons which did not demonstrate a climbing fiber discharge increased activity during preshapes. In contrast, most, but not all, Purkinje cells identified by the presence of complex spikes were inhibited during some phase of the task. Complex spike activity did not appear related in any way to the task performance. Several of the unidentified neurons demonstrated significant correlations between discharge frequency and other rates of change or with velocity parameters. The cerebellar vermis has been known to be involved with the processing of various sensory information and recently with the control of smooth pursuit eye movements. To further elucidate the relationship between the two, we have performed experiments with the various paradigms employed. All units in our population exhibited discharge modulations which reflected the velocity of smooth pursuit eye movements. In addition to having this eye velocity component, some units were also found to be responsive to retinal image motion. When eye movements were minimal or absent, the movement of the background pattern was associated with activity changes reflecting background movement velocity or with a direction dependent increase in firing whereby movement per se, rather than velocity, was the determining factor behind peak firing rate. Modulation in unit activity was observed in some units in conjunction with the movements of the test spot occurring while the monkey fixated the stationary red spot. As the velocity of test spot movement increased, the peak firing rate exhibited by these units also increased when the movement was either to the right or to the left. Modulation of unit activity was observed in one cell when the test spot moved in the visual field. Sinusoidal test spot movements, unit activity was modulated in phase with the changes in the retinal slip velocity associated with the test spot movement. With these cells, the same unit of retinal slip and eye velocity signals implicate the presence of neural correlates of target velocity signals. Among the units which were modulated by retinal slip velocity changes, some were also responsive to slippage of the entire visual field across the retina. In these units, was it again the velocity or the direction of movement which were the parameters related in unit discharges. Visual signals within the vermis, therefore, appear to convey 1) velocity information with respect to moving visual stimuli in general or 2) velocity information concerning discrete targets (spots) and directional information concerning movement of non-discrete images (background) across the retina. The target velocity signals would be most important to the extent that lobules VI and VII of the vermis play in the control of oculomotor function. Supported by NIH Grant EY01051.


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VISION STABILIZES FASTIGIAL NYSTAGMUS. T. Vilis and J. Hore.

The cerebellopontine system in the rat; an autoradiographic and phoretic injection system. After a 24 hour survival period, fro­

Thought the deep cerebellar nuclei. Projections arising from the lateral subdivisions of the pontine nuclei including the dorsal pedun­

ing input from the lateral cerebellar nucleus. The medial nucle­

The rate of drift was independent of eye position. Vision stabi­

In 3 monkeys in which this was studied, temperatures above a critical value produced a destabilizing effect on the tonic balance between the vesti­

The cerebral cortex, in contrast to the cerebellum, is a highly developed part of the brain. It is responsible for many of the higher functions of the human brain, including thought, language, and voluntary movement. The cerebral cortex is divided into two hemispheres, each of which is further divided into several lobes. The lobes are named for the areas of the body they represent: the frontal lobe, the parietal lobe, the occipital lobe, and the temporal lobe. The frontal lobe is involved in higher-level cognitive functions such as decision-making, reasoning, and planning. The parietal lobe is involved in sensory functions such as touch, temperature, and pain. The occipital lobe is involved in vision, and the temporal lobe is involved in hearing and language functions.

The cerebellum is a structure at the base of the brain that is involved in the regulation of voluntary movement and posture. It receives input from the cerebral cortex, the spinal cord, and other parts of the brain, and sends output to the spinal cord and other brain areas. The cerebellum is divided into three parts: the anterior lobe, the middle lobe, and the posterior lobe. The cerebellum is important in the control of muscle tone, balance, and coordination. It is also involved in the processing of sensory information and the formulation of motor plans.

The basal ganglia are a group of structures located at the base of the brain, consisting of the caudate nucleus, the putamen, and the globus pallidus. The basal ganglia are involved in the regulation of movement and are important in the control of voluntary movement. They receive input from the cerebral cortex and the thalamus, and send output to the thalamus and other parts of the brain. The basal ganglia are important in the control of muscle tone, balance, and coordination. They are also involved in the processing of sensory information and the formulation of motor plans.

The thalamus is a structure located at the base of the brain, just above the cerebral cortex. The thalamus is involved in the transmission of sensory information to the cerebral cortex, as well as the regulation of movement. It receives input from many parts of the brain and sends output to the cerebral cortex and other brain areas. The thalamus is important in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.

The hypothalamus is a structure located at the base of the brain, just above the thalamus. The hypothalamus is involved in the regulation of body functions such as appetite, thirst, and temperature. It also plays a role in the control of movement and posture. The hypothalamus is important in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.

The reticular formation is a network of neurons located at the base of the brain, just above the thalamus. The reticular formation is involved in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.

The sensory system is the part of the nervous system that processes information about the environment. It receives input from the sensory organs, such as the eyes, ears, skin, and muscles, and sends output to the brain and spinal cord. The sensory system is important in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.

The motor system is the part of the nervous system that controls voluntary movement. It receives input from the cerebral cortex and sends output to the muscles and glands. The motor system is important in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.

The autonomic nervous system is the part of the nervous system that controls involuntary functions such as heart rate, blood pressure, and digestion. It is divided into two parts: the sympathetic nervous system and the parasympathetic nervous system. The sympathetic nervous system is involved in the "fight or flight" response, while the parasympathetic nervous system is involved in the "rest and digest" response. The autonomic nervous system is important in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.

The endocrine system is the part of the body that produces hormones, which are chemical messengers that regulate the activities of other parts of the body. The endocrine system is involved in the control of voluntary movement and posture. It is also involved in the processing of sensory information and the formulation of motor plans.
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Two in vitro brain stem preparations have been developed. First, using our cerebellar slice technique (Llinás & Sugimori, Soc. Neurosci. Abst. 4, 1978), sagittal slices were prepared from guinea pig brain stems, which allowed direct recording from identified neurons in the inferior olive and in several reticular and motor nuclei. The second preparation consisted of whole brain stems comprising the levels from the C1-bulbar junction to the inferior colliculus. Brain stems were perfused with modified Ringer's solution through the basilar artery following ligation of both vertebral arteries. While the slice preparation could survive for >48 hrs after isolation, the brain stem survived for close to 7 hrs. Both preparations permitted the study of electrical properties of inferior olive cells. These neurons were seen to generate, through antidromic or direct stimulation, a fast action potential followed by a prolonged calcium-dependent spike. This spike, most probably of dendritic origin, triggered a potassium conductance change which generated a large (10-15 mV) after-hyperpolarization lasting for 200-250 msec. The initial fast spike could be blocked by 10⁻⁵ tetrodotoxin or by removal of external sodium from the perfusion medium. The calcium dendritic spikes were not blocked by these procedures but could be prevented either by application of cadmium, cobalt, or manganese salts, or by removal of calcium from the superfusion fluid. In addition to the above, hyperpolarization of 5-10 mV beyond resting level (70 mV) revealed a second calcium current of probable somatic origin. This voltage-dependent calcium conductance, which was inactivated at the normal resting potential, was capable of generating a calcium spike at a lower threshold than the one required for generating sodium-dependent action potentials. However, it was not blocked by tetrodotoxin or removal of sodium, but was blocked by cadmium, cobalt, or manganese. In addition, and as opposed to the dendritic calcium spikes, it showed a marked refractoriness indicating that these two $g_{Ca}$ differ in their properties and location. Thus, during the normal firing, sodium-dependent action potentials activated dendritic calcium action potentials. In turn, the large calcium-dependent potassium conductance generated the prolonged after-hyperpolarization, and as a rebound from this hyperpolarization a calcium-dependent somatic spike, which restarts the whole cycle. The inferior olive cell is therefore capable of behaving as a single cell oscillator if modulated to the proper membrane potential levels. Finally, simultaneous penetration of a pair of inferior olive cells allowed direct demonstration of electrotonic coupling between these neurons. (Supported by USPHS grant NS-13742)
CEREBRAL CORTEX
This experiment investigated the effects of lesions of the Medial Frontal Cortex (MF) and the Fornix (FX) on the retention and reversal of a non-spatial Object Discrimination and a spatial Location Discrimination. Acquisition training, prior to surgery, resulted in each rat learning one of the two discriminations. The Object discrimination was designed so that the rats learned to discriminate between two objects based on characteristics of the objects themselves. Neither the location of the objects in the test arena nor the turns required to approach them predicted the correct choice. The Location Discrimination was designed so that rats learned to discriminate between two objects based on their location within the test arena. Neither the objects nor the turns required to approach them predicted the correct choice. All rats learned a discrimination and performed well on various transfer tests designed to be sure they had used the appropriate choice strategy.

Retention testing began three weeks after the rats had received either 1) a lesion of the MF, 2) a lesion of the FX, or 3) a control procedure. Rats with lesions of the MF were unimpaired in the retention of both the Object and Location Discriminations. Rats with lesions of the FX, however, were impaired in the retention of the Location but not the Object Discrimination. Reversal training was begun after the rats had completed retention testing and each rat was given three successive discrimination reversal problems. Rats with lesions of the MF were impaired in both reversal tasks, as were rats with lesions of the FX. Rats with FX lesions, however, were significantly more impaired in reversal than rats with MF lesions.

In summary, reversal of the discriminations was impaired regardless of the modality tested: frontal and hippocampal system damage. This provides no support for the theories which suggest that these brain areas are differentially involved in spatial (as compared to non-spatial) tasks.

Retention was unimpaired following MF lesions while only the retention of the Location Discrimination was impaired following FX lesions. This suggests that the retention in the spatial modalities may be particularly sensitive to damage in the hippocampal system.


Past evoked potential and lesion studies attempted to determine the location of neurons whose axons project in the pyramidal tract (PT). Results indicated that all PT-neurons reside in the rostral third of the cerebral cortex, with maximum concentration in motor and somatosensory cortex. However, both methods yield only very approximate surface maps of underlying "PT-cortex" and do not identify the specific cortical layers of the neurones. We therefore used the horseradish peroxidase (HRP) technique to determine quantitatively the distribution in cortex of the total population of PT-cells and to study cell morphology. In anaesthetised cats, one pyramidal tract was microdissected off the ventral surface of the medulla. A complete cross-sectional cut of the trunk was made and HRP applied to the proximal PT-stump. Thirty hours later, the cats were perfused and the brains removed. Whole-brain frozen sections were cut (frontal and sagittal planes), reacted with DAB and stained with cresyl-violet. Using dark-field microscopy and an x-y plotter (controlled by the microscope stage), the brain sections were outlined on paper and each labelled neuron in the section plotted as a dot. The total cortical region containing PT-cells was divided into eight subregions and the percent of PT-cells determined in each. Subregions 1, 3 and 8 are surface cortex: # (containing 6-12% of PT-cells) extends caudal to the cruciate sulcus up to the lateral sulcus, # (7-8%) covers cortex laterally adjacent to #3. Subregions 2, 4, 5, 6, and 7 represent tangential cortex: #2 (5-10%) is the lateral bank of presylvian sulcus, #4 (16-22%) the ventral bank of the cruciate sulcus (Brodmann's area 6), #5 (30-35%) the dorsal bank of the cruciate sulcus (Brodmann's area 4), #6 (2-5%) the dorsal bank and #7 (1-4%) the ventral bank of the lateral sulcus. Thus, the HRP-technique revealed that only 30-40% of PT-cells is in surface cortex (30-40% of PT-cells is in surface cortex). However, projection of all labelled PT-cells to overlying surface cortex yields an area of "PT-cortex" in rough agreement with that of the older studies. PT-cells were also found in a new cortical area, the lateral bank of the presylvian sulcus, thus far not considered part of "PT-cortex". The cruciate sulcus marks the point of emergence from the callosum, below surface cortex. The hidden banks of this sulcus contain greatest concentration of PT-cells. Supported in part by NSF grant BNS 78-06953 and NIH grant RR-00166.

ARCHITECTURE OF MURINE THALAMOCORTICAL CONNECTIONS. V.S. Caviness and D.O. Frost. Eunice Kennedy Shriver Ctr. for Mental Retardation, Waltham, Mass. and Inst. of Anat., U. of Lausanne, Lausanne, Switzerland.

The pattern of thalamocortical projections was studied in adult mice. In each animal a lesion was made in either the anterior, lateral, medial, or posterior (but not in the medial or anterior) thalamus. Rats were sacrificed and their brains stained with the Fink-Heimer method. Sections from unoperated brains stained with cresyl violet or the Fink-Schneider silver stain. The terminal silver grains in layers I and IV. In sagittal sections two columnar configurations were seen in ipsilateral area 18. Since the axis of these projections was in the sagittal plane, they could not be discerned in coronal sections. A light projection to cortical layer I in the superior lip of the rhinal sulcus was also observed. In area 17 contiguous to the injection site a density of silver grains was found with the heaviest concentration in layers I, II, III, and VI. Area 18 and the medial border of 17 have commissural connections to the contralateral cortical homologue, whereas ipsilaterally these areas connect with the contiguous area 17 and area 18. There also appeared to be connections between area 18 and 17 specifically through the cingulate cortex and the cortex in the superior bank of the rhinal sulcus.
360 AUDITORS "ASSOCIATION" CORTEX AND DELAYED VISUAL MATCHING.

Five monkeys (M. fascicularis), previously evaluated on a delayed cross-modal symbolic matching task, have been trained to perform a delayed visual identity-matching task in which intratrial delays are varied from trial to trial by titration. The group includes three animals with lesions of the left superior temporal gyrus (so-called auditory association cortex), one with a unilateral lesion of the primary auditory cortex, and one unoperated control. In a previous report (Dawson, et al., J ACoust Soc. AMER, 58:896, 1975), the operated and controls were required to pair, after unfilled delays, an acoustic sample (a tone or a noise) with a visual match (red or green). Animals in which the left superior temporal gyrus had been removed could not achieve the delay durations they had achieved preoperatively on this task. In the present experiment, as would be expected, delays achieved for strictly visual matching are longer than those attained on the cross-modal task. Ablation of the auditory "association" cortex does not, therefore, affect performance on a delayed visual identity-matching task.

In addition, the monkeys are being tested on a delayed visual symbolic matching task in which a cross and a triangle are presented with the same red and green, respectively. This experiment will demonstrate whether ablation of the left superior temporal gyrus affects symbolic matching in the visual modality. Deficits in performance on the symbolic visual task attributable to prior removal of the auditory "association" cortex would show that the observed effects could be due to a disruption of a second-order association of the match with the sample.


Hoarding, a complex sequenced motor behavior, appears during the fourth week of life in the golden hamster. Since hoarding is dependent on the integrity of medial prefrontal cortex (MPFC) or medio dorsal projection cortex (Shelley and Kolb, 1977), we have initiated studies to determine whether developmental changes in the connections of this region might be associated with the emergence of this behavior.

Small volumes (.01-1 µl) of 3H VRP were injected unilaterally through glass micropipettes (tips 30-60 µm) into the MPFC of hamster pups. The animals were sacrificed after 24 hours and their brains processed with the TMB procedure of Mesulam (1976). We describe here preliminary results from 8 brains (2 each at 10, 20, 25 and 33 days of age) with relatively large injections involving areas 8 and 24 from the frontal pole to the level of the genual corpus callosum, anterior commissure and internal capsule. Few labeled cortical neurons could be found outside of the injection site. Although callosal projections were heavily labeled by Day 20, ipsilateral cortical projections were sparse with only a few labeled neurons present in several parietal and temporal cortical areas. Ablation of ipsilateral cortical neurons was first seen after MPFC injections in Day 25 and 33 animals in layers V and VI of areas 35 and 41. The results suggest whether the difference in cortical labeling seen after injections at different ages reflects a difference in uptake or transport mechanisms or a true difference in neuronal connectivity is currently being investigated.

This research supported by NIH grant 1S516 to C.M.L. and a NSF predoctoral fellowship to J.E.C.


Rats, like many animals, are able to distinguish places they have visited from those they have not. A number of investigators, using the radial maze (Olton and Samuelson, JEP: AB P 2: 97, 1976), have shown vision is an important component of this ability. In our previous experiments it was found that naive rats blinded by enucleation made significantly more errors than naive sighted rats before reaching criterion performance on an eight-arm radial maze. Experience rats exhibited only slight and temporary decrements in performance after blinding.

The present experiment was designed to investigate the role of the visual cortex (areas 17, 18 and 18a) in the maintenance (retention) of performance on the radial maze. Following training to criterion on the eight-arm radial maze, rats received either sham operations (group C), bilateral eye enucleations (group E), lesions of the visual cortex (group VC), or eye enucleations plus lesions of the visual cortex (group EVC). While group E showed a slight but significant performance decrement relative to group C, the other two groups, with lesions of areas 17, 18 and 18a, each showed a massive deficit. This large deficit was observed even in group EVC in which both the eyes and the visual cortex had been removed.

These results suggest that the visual cortex not only plays an important role in the maintenance of accurate radial-maze performance in sighted animals, but that its integrity is necessary for the maintenance of criterion performance in blind animals.


Simultaneous, optical assessment of tissue NADH-level, blood flow and blood volume was performed in the brain cortex with dimension of the order of 1 mm2 is presented. Measuring on the cortex covered by a grid with thickness 166 nm and diameter 500 µm, respectively. The tissue NADH-level was measured by measuring the NADH-fluorescence at 450 nm (according to Chance et al. (Sci. 174:499-508, 1971)). It is corrected for the hemodynamic artifact following the method of Habib et al. (J. Appl. Physiol. 41:480-486, 1976). The absolute values of blood flow and blood volume were determined at the same microarea by the microelectrometric indicator dilution method of Eke et al. (Am. J. Physiol. 236(5):H759-768, 1979), which analyzes the optical density of the tissue at 366 nm during induced tissue hemodilution. A computer controlled microfluororeflectometric system measures all of these parameters automatically, providing a value for the corrected NADH-level, blood flow and blood volume every 30 second. In this way an accurate correlation of these parameters with respect to time can be made, even during extremely rapid events of tissue metabolism and blood supply (see shaded area in the figure). As the neuronal tissue and its supplying microvessels are in contact only at the microcircular level, and since we are monitoring events at this level, our method can provide needed data on the interdependent and interrelated control of tissue metabolism and blood supply in the brain cortex. The non-invasive feature of the methodology seems essential in such a study.

Figure: Cerebrocortical microregional corrected NADH-level (mrNADH), blood flow (mrCBF) and blood volume (mrCBV) before, during and after an induced epileptic seizure. mrNADH is a measure for the local NADH-fluorescence corrected for the hemodynamic artifact following the method of Habib et al. (J. Appl. Physiol. 41:480-486, 1976). The absolute values of blood flow and blood volume were determined at the same microarea by the microelectrometric indicator dilution method of Eke et al. (Am. J. Physiol. 236(5):H759-768, 1979), which analyzes the optical density of the tissue at 366 nm during induced tissue hemodilution. A computer controlled microfluororeflectometric system measures all of these parameters automatically, providing a value for the corrected NADH-level, blood flow and blood volume every 30 second. In this way an accurate correlation of these parameters with respect to time can be made, even during extremely rapid events of tissue metabolism and blood supply (see shaded area in the figure). As the neuronal tissue and its supplying microvessels are in contact only at the microcircular level, and since we are monitoring events at this level, our method can provide needed data on the interdependent and interrelated control of tissue metabolism and blood supply in the brain cortex. The non-invasive feature of the methodology seems essential in such a study.

Figure: Cerebrocortical microregional corrected NADH-level (mrNADH), blood flow (mrCBF) and blood volume (mrCBV) before, during and after an induced epileptic seizure indicated by burst activity in the fronto-parietal ECoG (shaded area). After this time the ECoG activity returned to normal. Pentetrazolum was injected to induce the seizure in a dose of 27 mg/kg body weight into the cerebral circulation via the ipsilateral lingual artery.
BLANCA ESPLA-BOGNET

AN EXAMINATION OF COMMISSURALLY PROJECTING NEURONS IN THE PRIMARY SOMATOTAXIC CORTEX. S.H.C. Hendry* (SPON: E.G. Jones. Washington University School of Medicine, Department of Anatomy and Neurobiology, St. Louis, MO 63110).

Commissurally projecting neurons in the first somatosensory area (SI) of the cynomolgus monkey have been examined by light and electron microscopic methods. Neurons have been identified as commissurally projecting (HRP) from the contralateral SI in two ways:
1) In histochromically processed material, by the presence of a reaction product in cells which had retrogradely transported horseradish peroxidase (HRP).
2) In Golgi processed material, by the characteristic size, shape and location of these cells. The fine structure of cells identified in either way has been preserved in material in which thalamocortical terminals have been "labeled" by retrograde degeneration.

Commissurally projecting neurons in SI are pyramidal in shape and are located predominantly in layer IIB. Their basal dendrites, however, are present within layer IV, where they ramify extensively. Axon collaterals of commissurally projecting neurons can be seen to arise in layers IIb and IV. On the somata and proximal dendrites of these neurons a small number of symmetric synapses are present. Anomocytic synapses arise, in part, from axons which form two or more en passant contacts with a single cell. By contrast with the somata and dendrites, the axon hillock-initiation segment region receives a large number of symmetric synapses. Apparently several types of terminals end upon this region, each at a characteristic point along the length of the initial segment.

Evidence suggesting that some commissurally projecting neurons receive monosynaptic thalamocortical inputs will be presented.
THALAMIC PROJECTIONS TO LAYER I OF RAT NEOCORTEX. Miles Herkenham.

In previous autoradiographic studies of thalamocortical connections, I have shown that the ventromedial nucleus (VM) projects to layer I of widespread neocortical regions (JCN, 1979, 18, 457), whereas the ventral posterior lateral (VPL) and ventral posterior medial (VPM) nuclei have revealed a region of thalamus located for the most part laterally adjacent to the intralaminar nucleus, that projects primarily to layer I. The region comprises the ventral anterior (VA), posterior (PO), and magnocellular medial geniculate (MGM) nuclei. The projections from this region differ in two respects from those of VM: they are directed to more restricted cortical layers, and they are not confined to the boundary layer of termination. The dorsal and medial parts of VA, project far beyond the limits of the traditionally accepted terminal zones in the motor areas, into layers I and VI of parietal and occipital areas. Termination in the superficial half of layer I of visual cortex is remarkably dense. PO projects to layers I and V of parietal areas: the heavy layer I band occupies a deeper location, in Ib, whereas the secondary band is situated in the cell poor zone, Va. MGM projects to the outer two-thirds of layer I, and to a lesser extent to other portions of auditory cortex and more rostral levels. Depth profiles indicate that there are no sharp boundaries of terminal layering, even in layer I, after the VM injection.

Still other thalamic nuclei project to layer I, but not as heavily as to deeper layers. These include the mediodorsal, lateral dorsal, ventral posterior lateral (VPL), and ventral posteromedial (VPM), whose primary layer of termination is found in layers III and IV. Interestingly, several thalamic nuclei have terminations whose laminar patterns are area-dependent in the VM nuclei. The VM-Lamina III-V terminal pattern is found in the motor cortex, but is not as well represented in the visual cortex.

The laminar distribution in motor cortex, but more represented in the visual cortex, is shown to be less extensive than those of VM: they are directed to more restricted cortical layers, and they are not confined to the boundary layer of termination. The dorsal and medial parts of VA, project far beyond the limits of the traditionally accepted terminal zones in the motor areas, into layers I and VI of parietal and occipital areas. Termination in the superficial half of layer I of visual cortex is remarkably dense. PO projects to layers I and V of parietal areas: the heavy layer I band occupies a deeper location, in Ib, whereas the secondary band is situated in the cell poor zone, Va. MGM projects to the outer two-thirds of layer I, and to a lesser extent to other portions of auditory cortex and more rostral levels. Depth profiles indicate that there are no sharp boundaries of terminal layering, even in layer I, after the VM injection.

A spectrum of highly reproducible malformations can be induced in rats with pre- or early postnatal x-irradiation, and such is known about their morphogenesis (Hicks and D'Amato, 1978). A spectrum of highly reproducible malformations can be induced in rats with pre- or early postnatal x-irradiation, and such is known about their morphogenesis (Hicks and D'Amato, 1978). A spectrum of highly reproducible malformations can be induced in rats with pre- or early postnatal x-irradiation, and such is known about their morphogenesis (Hicks and D'Amato, 1978). A spectrum of highly reproducible malformations can be induced in rats with pre- or early postnatal x-irradiation, and such is known about their morphogenesis (Hicks and D'Amato, 1978). A spectrum of highly reproducible malformations can be induced in rats with pre- or early postnatal x-irradiation, and such is known about their morphogenesis (Hicks and D'Amato, 1978).
In an effort to determine movement-coupled activities. Neuron activities were further tested in a task in which two different sizes of the movement of the same direction were presented. Size-dependent activity appeared in delay period in PF neurons but not in PTNs. Higher rates correlated to larger movement was observed in 26 and those to smaller one were in 8 PF neurons. 

It is concluded during delay period of the delayed-response task that in PF neurons both direction and size of the movement are differentially encoded and that in both PF neurons and PTNs there are activities capable of facilitating movement-coupled activations of PTNs.


A projection from the cortex within the concavity of the arcuate sulcus, the "frontal eye field," of the monkey, has been previously described by Kuypers and Lawrence (1967) and Astrup (1971) using silver methods, and more recently by Kume, Astrup and Wurtz (1979) using the autoradiographic method. We have found that the use of our horseradish peroxidase gel (Griffith et al. Br. Res. in press) in combination with the TMB peroxidase process produces a clear delineation of both anterogradely and retrogradely transported enzyme, such that one can virtually map the environment or (b) limited to a more restricted area in the prefrontal cortex. 

In a previous report, we described cortical columnar (modular) organization, using the (4c)-2-deoxyglucose (DG) technique, in primary and secondary somatosensory areas (S1, SII) of anesthetized, paralyzed Cynomolgus monkey. This animal was stimulated with a corneal brush stroke (reported here in 1979). Our current work has evaluated unstimulated animals and additional monkeys, varying the proximodistal location of the stimulus. Although monkeys were located either from the frontal interhemispheric joint to the tip of the left index finger or on the anterior midline of the left forearm (1.5 cm).

The anterograde and retrograde labeling were after Sokoloff (J. Neurochem., 28: 1977). We found that the size of modules and basic organizational pattern of metabolic activity to compare with that of our initial report in S1 and SII. Contralateral modules in a and b averaged 360 µ, and in area 1, 350 µ. The modules for these two recently stimulated monkeys were (a) confined to a more restricted cortical topological area (b) decreased in number and (c) primarily in areas 1 and 2. This labeling corresponded to somatotopic representations in S1 as previously described. In the control animal there was a paucity of modules in areas 3a, 3b, 1 and 2. In four of our monkeys examined there was additional labeling in other cortical areas. These included areas 5 and 7, area 4 adjacent to 3a, the inferior bank of the lateral fissure, the insula, and both banks of the superior aspect of the superior temporal sulcus (STS). Activity in these areas was columnar in nature, but the modules were less regular in temporal extent than those in other areas.

Recent findings that the motor cortex PTN activity is modulated in response to neuronal activity in the frontal eye field (FEF) reveals both movement-related and movement-related PTN activities. Movement-elicited activity in a central area of the FEF was present in the monkey, has been previously described by Kuypers and Lawrence (1979). Our current work has evaluated unstimulated animals and additional monkeys, varying the proximodistal location of the stimulus. Although monkeys were located either from the frontal interhemispheric joint to the tip of the left index finger or on the anterior midline of the left forearm (1.5 cm).

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375 Brightness Encoding in Cells of Area 17 in the Awake Behaving Rhesus Monkey. Maguire, W. M.,* Baizer, J. S.,* and Weiss, C.,* Division of Neurobiology, Department of Physiology, School of Medicine, SUNY, Buffalo, NY 14226.

The manner in which such stimulus dimensions as orientation, direction and wavelength affect the responses of the cells of striate cortex has been described in detail in recent years. Little is known about how these cells respond to movement, which is a group of stimuli that provides information about luminance at higher stimulus luminances. The responses to these stimuli were record-


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CEREBRAL CORTEX

376 CORTICAL CELL RECORDING AFTER 3 YEARS WITH CHRONICALLY IMPLANTED MICROELECTRODES. J.S. McIntosh, E.M. Schmidt and M.J. Bak. Lab. of Neural Control, MINDS, NIH, Bethesda, MD. 20205.

Chronic microelectrodes have been implanted in the precentral cortex of a number of monkeys for long term monitoring of single unit activity. One chronic implant was 3 years and 49 days old on the day of sacrifice of two of the eleven implanted electrodes were still recording neuronal activity. Examples of this activity are shown in Fig. 1.

During the course of the implant a number of different cells were recorded from a single electrode, indicating that the electrodes were moving in the brain. The movement was usually very slow, allowing the same cell to be recorded for many days or months. The criteria for classifying the neuronal activity as originating from the same cell were: (a) the wave shape was similar from day to day, (b) sensory field was the same, (c) the cell maintained the same firing pattern during trained movements, and (d) when the cell was operantly conditioned to fire at specific rates the monkey made the same movements on succeeding days. The longest recording from the same cell was 108 days.

Vapor deposited Parylene-C was used to insulate the iridium microelectrodes and gold lead wires. With three of the eleven electrodes the insulation remained intact over the entire period of implantation. Pin holes through the insulation along the electrode shafts developed in the other electrodes. When the electrode impedance dropped due to insulation breakdown, neuronal activity was usually lost due to the shunt path. Scanning electron micrographs of some electrodes show surface defects in the insulation that may have been due to procedures in initial electrode preparation.

Histological sections of the brain tissue revealed little or no reaction to Parylene coated electrodes. From these results it appears that very long term recordings from the nervous system are possible.


Recent neurophysiological studies in behaving monkeys indicate that the dorsolateral prefrontal cortex contains visual areas of which the visual cue stimuli, but relations between physical parameters of the stimulus and neuronal responses have not yet been examined systematically. This study attempted to determine the visual response properties of PF neurons, more specifically, their visual receptive field (RF) size.

Four rhesus monkeys were trained to perform a visual fixation task; fixing their eyes for 0.5-5 s on a small circular spot (0.5°, 1.0 cd/m²) at the center of a tangent screen (1.5 X 1.2 m). The trial was started when the recording began and continued for a few minutes. After a few minutes of waiting period, a spot appeared and then 0.5-3 s later, a slit stimulus (2.5 cd/m²), unrelated to reinforcement, was presented for 0.5 s. Within 0.5 s after the slit, spot brightness was changed. Releasing his hand from the lever, he was rewarded. A total of 197 neurons was sampled from the dorsolateral PF cortex while the monkey was performing a task in which a visual stimulus of slit size of relatively large size (6°-40°/s, 3° X 30°) was routinely presented in the contralateral visual field. Seventy neurons showed clear changes of their discharge rates in response to the fixation spot with latency of 100-300 ms. Of these, 40 neurons responded also to extrafoveal slit stimuli with similar latency. The slit was moved away from the RF center. Only 5 neurons showed a clear surround inhibition. In 16 neurons the RF was contralaterally to the slit recorded side and in 2 it was ipsilateral. In 47 neurons it expanded from contralateral to ipsilateral side. As for topography of recorded neurons, rostrally located neurons tended to have larger RF. Responses to stationary and moving slits were compared in 36 neurons, and 7 showed stronger responses to the moving slit (6°-12°/s) than to stationary one. Different selectivity of response to the direction of movement was found in 5 neurons.

Thus, dorsolateral prefrontal neurons have relatively large visual receptive fields. It is projected mainly from the contralateral hemifield.

378 AFFERENT VISUAL SIGNALS FOR DIRECTED VISUAL ATTENTION. Brad C. Motter* and Vernon B. Mountcastle, The Johns Hopkins University School of Medicine, Baltimore, Md.

Single neuron studies were made in the cortex of the inferior parietal lobule (area 7) in 8 hemispheres of macaque monkeys trained in tasks requiring maintenance of directed visual attention. Area 7 contains a large number of sets of cortical neurons, including those active during prefrontal and manipulation with arm and hand; those active during fixation and detection. Cells of all these sets show clear changes of their discharge rates in response to stimuli within the monocular response areas that commonly extend towards the contra-lateral hemifield.

Further, 127 neurons responded only to the slit. The RF was determined in 35 neurons, using a 1° X 5° or 1° X 1° slit stimuli. In 21 neurons the RF was contralateral X 10° to 60° X 60° and in 28 neurons it was larger than 30° X 30°. Often it was difficult to draw a boundary line between responsive and unresponsive areas because these areas gradually weakens, and in 28 neurons it was ipsilateral. In 47 neurons it expanded from contralateral to ipsilateral side. As for topography of recorded neurons, rostrally located neurons tended to have larger RF. Responses to stationary and moving slits were compared in 36 neurons, and 7 showed stronger responses to the moving slit (6°-12°/s) than to stationary one. Different selectivities of response to the direction of movement were found in 5 neurons.

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Electrical stimulation mapping of human perisylvian cortex during standard tests of naming, reading, short-term verbal memory, single and sequential oral facial movements, and phonemic perception was undertaken during craniotomy under local anesthesia for resection of epileptic foci. The evoked changes in each of these tests were measured at 8-15 preselected sites in left brain of 5 patients and right brain of 3 patients. In each area, a 1500 wV stimulus threshold was set for the left brain dominant for language. Anterograde extracranial anteriority. The procedures used for obtaining informed consent for the research portions of this study had been reviewed in advance by the institutional biomedical sciences review committee in accordance with the applicable Public Health Service guidelines. The experimental operation, stimulation, and recording were performed on 5 patients, who had prior informed consent for the research portions of this study. The patients had severe aphasia and were 24-61 years old. All patients had been studied in the preoperative period and there were 2 with cerebral angiograms of the upper brain stem and 3 with brain stem measurements. The current was at the highest level that did not alter discharges (4.8mA).

The ability to mimic sequential oral facial movements and to perceive phonemes was altered by stimulation of the same left brain sites, at least 2 in each patient. This identifies a cortical sequential motor-phonemic perception language system that provides a link between speech production and understanding. This is an anatomic basis for the motor theory of speech perception (Liberman et al., Psychological Reviews 47:41, 1967). These sites are in perisylvian cortex of frontal, temporal and parietal lobes, an area that would be included in almost every cortical lesion that gives rise to persisting language disorder. Phonemic perception was also unaltered at sites 1 mm below and 3 mm lateral to the left brain dominant for language. Anterograde extracranial anteriority. The procedures used for obtaining informed consent for the research portions of this study had been reviewed in advance by the institutional biomedical sciences review committee in accordance with the applicable Public Health Service guidelines. The experimental operation, stimulation, and recording were performed on 5 patients, who had prior informed consent for the research portions of this study. The patients had severe aphasia and were 24-61 years old. All patients had been studied in the preoperative period and there were 2 with cerebral angiograms of the upper brain stem and 3 with brain stem measurements. The current was at the highest level that did not alter discharges (mean 4.8mA).

This research was supported by NIH Research Grant NS 40403, awarded by NIHDCS, PL/44451.

In several mammalian species electrical stimulation of discrete areas within the frontal cortex, the frontal eye fields, elicits discrete eye movements. Although the FEF's have been studied with a number of techniques, the involvements of these cortical areas in ocular movements remain unclear. In order to further elucidate their role in specific eye movements we are tracing the connections of the FEF's with subcortical structures using horseradish peroxidase (HRP) in conjunction with electron microscopic studies.

In cats lightly anesthetized with chloral hydrate, the FEF's are stimulated with 25-50 µA negative current via bipolar electrodes to produce a saccadic eye movement, whereafter 0.2-0.05 ul of 10-40% Sigma VI HRP is injected at the site of lowest threshold. The brains are processed for HRP after 48 hr. survival time utilizing a tetramethyl benzidine procedure similar to that described by Mesulam (1978).

Retrograde and anterograde transport of HRP can be traced to cortical and subcortical structures. In the frontal cortex retrogradely labeled cells appear primarily in laminae II and III of the contralateral FEF. Labeled cells are present ipsilaterally in restricted areas of the cingulate, suprasylvian and ecosylvian gyri.

Heavy retrograde and anterograde labeling is apparent in the ipsilateral claustrum. Significant numbers of cells are found throughout structures in the rostral basal forebrain, including the vertical limb of the diagonal band of Broca, the substantia innominata, the anterior hypothalamic area and the basal magnocellular amygdaloid nucleus.

In the ipsilateral diencephalon HRP is found in the dorsal thalamus and hypothalamus. The ventrolateral portion of the medial dorsal nucleus, the medial portions of the ventral anterior and ventral lateral nuclei, and the ventral medial thalamus all exhibit retrograde and anterograde transport. Retrogradely labeled cells are also seen in the lateral and medial areas of the hypothalamus.

Several large labeled cells are found in the suprageniculate nucleus and pretectal area, ipsilaterally. In the brain stem labeled cells are seen in the rostral tegmental nucleus, locus coeruleus and raphe nuclei.


In a previous study of smooth and sparsely-spined stellate cells in the rat visual cortex using a combined Golgi-electron microscope technique (Peters and Fairen, J. Comp. Neurol., 181: 129, 1978), it was shown that the axons of these neurons form asymmetric and inhibitory synapses. The post synaptic elements are various and include the cell bodies and dendrites of pyramidal neurons. However, this study provided no information about how such synapses one stellate cell can make with an individual pyramidal neuron, or how these synapses are. The opportunity to investigate this arose when a light microscopic examination of a Golgi impregnated preparation, which had been gold-tone for electron microscopy, revealed a stellate cell giving rise to some boutons which were apparently associated with an impregnated pyramidal neuron in layer III. On the basis of light microscopy we predicted that some of the axonal boutons of the stellate cell apparently opposed to the pyramids were forming synapses with it. Upon ultrastructural examination of the apposing boutons in serial thin sections, in which the profiles of both neurons were identified by their content of gold particles, the prediction was verified. By electron microscopy, the boutons observed in the light microscope resolved into two separate axon terminals when the thin sections were examined and nine axon terminals were found to synapse with the pyramidal neuron. Five terminals formed axosomatic synapses, one synapsed with the shaft of the apical dendrite, and three synapsed with basal dendrites. In addition we encountered a synapse between an axon terminal of the stellate cell and one of that cell's own dendrites.

DIRECT AND INDIRECT PROJECTIONS FROM THE MOTOR CORTEX TO THE INFERIOR OLIVE IN THE CAT. J.A. Saint-Cyr and J. Courville, Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec.

Injections of horseradish peroxidase (HRP, 20-30% Type VI, 1-1.5 µl) in the inferior olivary complex of 10 cats resulted in the retrograde labeling of cells in the motor cortex. The labeled cells were found in layer V and were more numerous ipsilaterally. They were present in the pyramidal layer and the deep subpyramidal layer of the cruciate sulcus and were sparsely distributed. A few were also present in the orbital, coronal and preoral gyril.

Injections of tritiated amino acids (³H-³-leucine [150-330 µCi], or ³H-³-lysine, ³H-³-lysine and ³H-³-amino acid mixture [200 µCi/µl], NEN, vols. 2-2.5 µl) were made in the cruciate region of 6 cats. Five injections involving the anterior sigmoid gyrus resulted in the labeling of the inferior olive. In the inferior olive, the caudal medio-lateral portion of the accessory olivary cell mass (MO) excluding subnucleus B were bilaterally labeled in two cases. The grain density was greater ipsilaterally than contralaterally. In three other cases, similar but very weak deposits were present. Previous studies had indicated terminations from the cortex in the rostral pricipal (PO) and dorsal accessory (DAO) olives (Sousa-Pinto and Brodal, Exp. Brain Res. 1969, 5: 364). Number of fibers of passage were observed to pass through the rostral PO and DAO in our material and the presence of terminations was equivocal. Further experiments using shorter survival times are planned in order to resolve this question.

In the mesencephalon deposits of silver grains over the nucleus of Darkschewitsch, the interstitial nucleus of Cajal and the rostrolateral medial of the red nucleus were present ipsilaterally. In four cases, the lateral corticospinal cell groups were previously identified as major sources of input to the rostral lateral MO and PO (Saint-Cyr and Courville, Neurosci. Abst. 1978, #160). It is suggested that the motor cortex may influence widespread areas of the cerebellar cortex via the climbing fiber system principally through these mesencephalic relays.

Supported by a grant from the Canadian MRC to the group in Neurological Sciences, University of Montreal.
The intrahemispheric connections of the primary somatosensory cortex (SI) and the secondary somatosensory cortex (SII) appear to be laminae specific. The SI and SII cells of origin appear to be laminae specific. The SI and SII cells of origin primarily arise from layers III and V. The intrahemispheric projections of MII originate predominately from layers II and III pyramidal cells. The results indicate that while there are reciprocal cortico-cortical connections between homofunctional areas of MI, MII, SI, and SII, there are no connections of MI and MII with SI and SII in terms of the topography of body representations, specifically, with regard to the connections of the same cytoarchitectonic representation in the different body representations (heterofunctional). The cortico-cortical connections were investigated using the horseradish peroxidase (HRP) retrograde transport technique in the raccoon. Electrophoretic pressure injections of 30-50X HRP were made into electrophysiological defined regions in Mi, MII, SI or SII in chloralose anesthetized raccoons. For following a survival period of 3-7-12 hours, the animals were intracardially perfused with a buffered aldehyde mixture. The brains were processed for HRP histochemistry using tetramethyl benzidine and dihydrochlorobenzidine as the chromogens on adjacent sections. Following an injection into MI forepaw/area 3a, labelled cell bodies were observed in SI forepaw/area 3a and SII while following an injection into SI forepaw area, labelled cells were found in MI forepaw area. Injections into MI trunk area resulted in reactive neurons in a homofunctional area of MI. The effects of MI and MII injections into the SII hindlimb region, HRP positive cells were found in MI hindlimb area. After an injection in SII hindlimb region, labelled cell bodies were found in MI hindlimb area, and MI positive cells were found in SII area. Following an injection into the distal limb area of MI labelled cells were found in MI hindlimb area; HRP positive cell bodies were also found in the inferior and superior banks of the suprasylvian sulcus, a region which corresponds to the boudary region between SI and SII. The intrahemispheric connections of MII origin appear to be laminae specific. The SI and SII cells of origin primarily arise from layers III and V. The intrahemispheric projections of MII originate predominantly from layers III and II, while the MI cells of origin largely arise from layer III. The cells of origin are distributed into clumps or clusters. Typically, a rather continuous band of labelled layer III pyramidal cells is observed interspersed by a rather continuous band of labelled layer II. The cells of origin are distributed into clumps or clusters. Typically, a rather continuous band of labelled layer III pyramidal cells is distributed in a single plane throughout the tangential dimension of the thalamus. In order to define the thalamic dependencies of each of these stripes, HRP was injected into one, separately, whereas their efferent connections were demonstrated autoradiographically following injection of tritiated leucine and-proline. Results indicate that the medial strip has reciprocal connections with the thalamic nuclei VC, VM, and PO, and the more lateral strips with VB and PO. Corticospinal fibers to the ventral horn originate only from the medial strip, whereas the spinal projections from the more lateral strips extend ventrally beyond the dorsal horn. The corticocortical projections from each of the three strips distribute bilaterally to a long sagittal zone of corticostriate fields; the three areas somewhat different but overlap widely. Corticocortical fibers from the medial strip project to the two, more lateral strips, and in each are distributed in a discrete vertical column. In addition, the medial strip projects to a narrow peripheral region of cortex which, as revealed by our previous HRP study, projects to the spinal cord. The two more lateral strips project to the medial strip and to the homomotor pattern. Controlateral (collapsed) associations mirror the ipsilateral ones but are greater heterotypy than previously suggested. The present findings fail to identify the medial cortical spinal strip as the motor and the two lateral strips as somatosensory. At any rate, the corticocortical projections of sensory-motor overlap may require a re-evaluation on the basis of overlap of projections from the medial and the two lateral strips in PO and Striathum, as well as the cortico-cortical interconnections. (Supported by USPHS grant I-POI-MH3154.)

CORTICOTECTAL CONNECTIONS IN THE GERBIL. H.B. Sherman*, V.S. Grinzelas, Jr., D.P. Ingle* (Jr.): (J.H. Williams). Brandeis Univ. and E.K. Shriver Ctr., Waltham, MA 02154.

The connections between the neocortex (NC) and the superior colliculus (SC) have been studied in the gerbil by a combination of two methods: horseradish peroxidase injected into the SC and tritiated amino acids injected into a majority of the cytoarchitectonic fields of the NC. The corticocortical projection arises widely from layer V of the occipital, parietal, temporal, and frontal fields. This extends as a projection zone to at least an area of the SC, and is the result of individual projections; by individual projections is meant a projection which arises in one, in some instances two or three adjacent complete cytoarchitectonic fields and is distributed in a single plane throughout the tangential dimension of the OC. Some projections in these planes are complementary, to some extent overlapping, in the radial dimension of the SC. Individual projections arising in occipital fields 17, 18a, and 19 project in overlapping succession to the deeper levels of the stratum griseum superficiale, stratum opticum and the superficial levels of the stratum griseum intermediale. Parietal fields 3 and 1, temporal fields 22, 36 and 41, medial cortical fields 8 and 24 and parietal fields 40 and 14 project in overlapping succession to the stratum griseum intermediale and stratum album intermediale. Finally, frontal fields 6 and 4 project to the stratum griseum profundum and stratum album profundum.

Projections which arise in the occipital fields and those arising in the frontal fields terminate in a continuous plane in the stratum griseum and stratum album of the SC. The remaining projections, by contrast, terminate in a "puffs and holes" pattern, i.e., a discontinuous pattern within the intermediate collicular strata.

Each projection appears to be topologically organized; the cytoarchitectonic boundaries of the fields of origin of each projection are directed to the margins of the SC. In some instances, adjacent points in the cortex on opposite sides of a common cytoarchitectonic border are in radial register with each other at the margins of the SC, i.e., the 17a, 18a/19 borders. As a consequence of this principle of alignment, homologous points in the separate visual cortical representations are probably in register with each other within the SC. In other instances, adjacent points on opposite sides of a common cytoarchitectonic border appear not to be in register with each other within the SC. This occurs where cytoarchitectonic borders probably separate projections from different cortical systems, e.g., at the 22/40a and 8/6 borders. These borders probably lie between fields associated with the AI and SII and the Eye field and MI cortical representations, respectively.
A MODEL OF CORTICAL CIRCUITRY IN THE TURTLE BASED ON MORPHOMETRIC ANALYSIS OF THALAMIC FIBER INPUTS AND THEIR CORTICAL CELL TARGETS


Evoked responses in turtle cortex to optic nerve or thalamic stimulation habituated rapidly when the stimulus intensities were increased more frequently than 0.5 Hz (Belekhova and Kosareva, '71). Our experiments provide a model that explains why habituation is the dominant cellular response in this neural system. We undertook this study to determine whether the ratio of the number of thalamic synapses to the volume of the thalamic terminals and cortical cells in equivalent volumes of cortex. Fourteen days after unilateral removal of the thalamus, we prepared the ipsilateral cortex for routine EM in 15 Pseudemys turtles. All thalamic fibers end within 100 μ of the cortical surface, containing round agranular vesicles and forming asymmetrical (type I) contacts. These contacts are on dendritic spines and are on dendritic shafts. Unisensory evoked responses were found for thalamic contacts with the scattered cells located in the molecular layer. We calculate that there are 6.15 million thalamic synapses in a 1 mm cube of turtle cortex. Morphometric analysis of the cells in an equivalent volume of cortex leads to an estimate of 15,000 cells in the main cell lamina and 400 cells in the overlying molecular layer. Golgi preparations show that neurons in the main cell lamina have numerous spines on their apical dendrites. These dendrites ascend through the molecular layer into the thalamic input zone. In contrast, the vast majority of the cells in the molecular layer have smooth (apomorph) dendrites. For this calculation, we assume that contacts on dendritic spines are onto main lamina cells (pyramidal cells) while those 14% on dendritic spines contact molecular layer cells (stellate cells). Given that 14% of 2.9 million thalamic contacts onto the 15,000 pyramidal cells and 0.86 million thalamic contacts onto 400 stellate cells. The final step in this calculation predicts surprising and counterintuitive results, namely, that there are 6X as many thalamic synapses on each stellate cell as on each pyramidal cell (5.29 million / 15,000 = 353 contacts/pyramid and 0.86 million / 400 = 2150 contacts/stellate). We hypothesize that stellate cells in the molecular layers are inhibitory interneurons that receive 6X more excitatory than inhibitory input per neuron, resulting in higher excitability of both cell types, but the secondary effect of stellate cell activation is to leave the pyramidal cells hyperpolarized and refractory, to form asymmetrical (type I) contacts.

PERFORMANCE OF NEOSTRIATAL MONKEYS WITH BILATERAL INFERTEMPORAL CORTEX LESIONS IN A SIMULTANEOUS VISUAL PATTERN DISCRIMINATION TASK DEMANDING OF ATTENTION. Henry V. Soper, Susan Twigg, Thomas Gillman and Donald B. Modrack, Deps. of Psychol., Physiol. and Brain Res. Inst., UC, Los Angeles, CA, 90024.

Some monkeys in two groups were trained on a visual discrimination task in which the task demanded of attention. The pre-operative performances of the monkeys remained stable over the course of training, and post-operative performances were not significantly different from pre-operative levels. Postoperative performances of monkeys were analyzed by a 2 x 2 (Group x Hemisphere) factorial design. The postoperative performances of the monkeys were not significantly different from pre-operative levels. The monkeys were then tested on a visual discrimination task in which the task demanded of attention. The pre-operative performances of the monkeys remained stable over the course of training, and post-operative performances were not significantly different from pre-operative levels. Postoperative performances of monkeys were analyzed by a 2 x 2 (Group x Hemisphere) factorial design. The postoperative performances of the monkeys were not significantly different from pre-operative levels.
SYNAPTIC PROFILES OF SPINY AND NON-SPINY STELLATE CELLS.

Thalamocortical (TC) afferents to the posteroomedial barrel sub-field (PMdSBF) of mouse SmI cortex synapse with several different types of neurons (Brezina et al., '72). The most frequent recipient of TC input are the spiny cells whose somata occur in layer IV and whose dendrites are mostly restricted to barrel hollows. The precise location of this study was to assess the locations of TC and other synapses onto the cell bodies and dendrites of a spiny and a non-spiny stellate cell of mouse PMdSBF cortex. The method used to identify TC axon terminals was lesion induced degeneration; a lesion was used to destroy the corticopetal projections from the nuc-leus ventralis posterior pars lateralis and the nucleus posterior thalami in a 2 month old C57 mouse. Lesion induced degeneration is generally considered unreliable for the identification of TC axon terminals because in most systems TC terminals degenerate over a varied time course such that some terminals have been phagocytosed before others have begun to degenerate. This is not true of terminals degeneration in mouse PMdSBF cortex where all axons of the terminal field of the terminals in layer IV neuropil degenerate simultaneously. A Golgi impregnated spiny stellate cell from layer IV of PMdSBF cortex was examined in the lesion area. Synapses of a neighboring non-spiny stellate cell was cut and electron micrographs were taken of each part of the spiny stellate cell and of a neighboring non-spiny stellate cell which was not impregnated, but which could be identified on the basis of its cytology and synaptic connections. These cells were then reconstructed from sections of appropriate thickness at a final magnification of 20,000 X. TC and synapses of unidentified origin are depicted on the reconstructions. About 100 of the asymmetric synapses onto the spiny stellate cell were from the thalamus; all of these synapses are with spines. By contrast, the non-spiny stellate cell receives a much larger share of its inputs from the thalamus and these synapses contact both its cell body and dendrites. An intriguing finding is that TC inputs are distributed in a non-random fashion over extensive regions of the spiny stellate cell. For example, the necks of 5 spines which receive TC input attach to one primary dendrite at intervals of 5-10 microns. Our interpretation of this periodicity is that it reflects the spacing of TC axonendritic "junctions" prior to the time of spine formation.

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SUBLIMINAL SYNAPTIC INPUT TO CORTICAL NEURONS AND ITS DETECTION BY THE MODIFICATION OF ANTIDROMIC RESPONSES. D. Wiggin* and P. Zarzeczki (SPON: C. Romero-Sierra). Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Collision-extinction testing can be used to investigate synaptic inputs to neurons whose axonal projections have been identified by antidromic activation. A serious limitation of this method is its inability to detect subthreshold synaptic events and it seems, therefore, to underestimate the extent of synaptic input (Zarzeczki et al., Exp. Brain Res. 33: 269, 1978). An alternative method of assessing synaptic input is suggested by the observation that subthreshold excitatory inputs to spinal motoneurons facilitate somatic invasion of an antidromic action potential (Broock et al., J. Physiol. 122: 429, 1953). In the two systems which we have examined, an apparent facilitation has been observed as a shape change in the extracellularly recorded antidromic spikes.

Subthreshold input was tested by microstimulation of the motor cortex (area 4γ) or by activating two or more pyramidal tract (PT) neurons of area 3a with a single stimulus of sufficient intensity to evoke an antidromic spike. Extracellular recordings were made from cortico-cortical and pyramidal tract neurons in cats lightly anesthetized with Nembutal. Stimulation of forelimb nerves was frequently followed by a change in the shape of the antidromic action potential, even in the case of neurons which did not fire in response to the peripheral stimulus. The change in the antidromic spike most often consisted of a clear decrease in the time from onset to peak. The decrease was especially evident for neurons whose antidromic spike was fractionated, in which cases the inflection of the rising phase was reduced or eliminated. We conclude that this decrease in time from onset to peak is a result of an increase in the excitability of the soma. Therefore, monitoring changes in the time from onset to peak of extracellularly recorded antidromic spike potentials is a more sensitive method of detecting synaptic inputs than collision-extinction testing. We have now used this method to investigate the patterns of sensory convergence upon identified populations of cortico-cortical and pyramidal tract neurons, as will be described in the following abstract.

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CHEMICAL SENSES: SMELL AND TASTE
CHEMORESPONSIVE NEURONS OF THE GOAT GENICULATE GANGLION. James C. Bouvreaux, Joseph Gravec* and Nga Kieu Hoang*. Sensory Sciences Center, University of Texas at Houston, TX 77025.

Single unit recordings were taken from taste neurons of the geniculate ganglion of the anesthetized goat. Neurons typically innervated more than one fungiform papilla (10 or more not uncommon). Almost all neurons exhibited spontaneous activity. 15% of this activity was usually multiplexed with peaks in the short interval range corresponding to fixed interval bursting (although intervals shortened as a function of order in burst). Neurons were stimulated with a taste stimulus, a carnivore and a food and herb series. Many nonresponsive units were encountered. Amino acids in general were poor stimuli but monosodium salt was potent. Carrots were often potent stimuli. At least three different neural groups could be delineated on the basis of responses to chemicals. These groups also differed, in part, in latency to electrical stimulation, area of tongue innervated, and spontaneous and evoked discharge patterns. The two large fiber groups innervated all parts of the tongue, while the smaller fiber group innervated only the back. One of the large fiber groups (the Brønsted acid responsive group) could be compared with the neural groups described in the carnivore.

TASTE INTERACTIONS IN MIXTURES OF SUCROSE WITH NaCl AND SUCROSE WITH QHCl. Linda M. Bartoshuk. John B. Pierce Foundation Laboratory and Yale University, New Haven, CT 06519.

When taste substances are mixed, their tastes change in intensity even when there are no chemical interactions among the substances. These mixture interactions are complex and no attempt to formulate a set of taste mixture laws has yet been completely successful. One variable not considered in earlier mixture studies is the shape of the psychophysical function that describes perceived intensity versus concentration. When substances with similar tastes are mixed, the interactions are highly dependent on the shapes of these functions. For example, mixtures of substances with psychophysical functions that are compressed (i.e., successive increments in concentration produce smaller and smaller increments in perceived intensity) show suppression (i.e., the perceived intensity of the mixture is less than the sum of the perceived intensities of the component). This is not particularly surprising since mixtures of substances with the same taste quality would be essentially the same as higher concentrations of a single component if the substances act through a common receptor mechanism. There is less reason to expect interactions in mixtures of substances with different taste qualities to be dependent on the shapes of psychophysical functions, yet some show such dependence. The results of a series of studies show that mixtures of quinine hydrochloride (QHCl) and sucrose (of equal perceived intensity when unmixed) show interactions that can be related to the shapes of the unmixed psychophysical functions. That is, the more compression shown by the psychophysical function of a component, the more suppression shown by the substance in the mixture. On the other hand, in similar mixtures of NaCl and sodium chloride (NaCl), the bitterness of QHCl is always suppressed more than the saltiness of NaCl no matter what the shapes of the psychophysical functions. These two mixtures can also be distinguished by a cross-adaptation experiment. In general, if the tongue is adapted to one component of a two component mixture, then the mixture will taste like the other component down when it is unmixed (i.e., if the mixture suppression exerted by a component is released when that component is "removed" by adaptation). This release of mixture suppression fails to occur for QHCl in mixtures of QHCl and NaCl tasted after adaptation to NaCl. All of these experiments taken together suggest that the mechanisms underlying mixture interactions for these two classes of mixtures are not the same and that bitterness may be at least partially suppressed in mixtures of QHCl and NaCl at the tongue surface.


We previously reported that response characteristics of taste neurons in the sheep solitary tract and nucleus change during fetal development (Science 202, 535-537, 1978). When the tongue was stimulated with 0.5M NH₄Cl and NaCl, solitary neurons in fetuses <144 days of age only responded to NH₄Cl and KCl, whereas neurons in older fetuses, lambs and adults responded to all four salts. To determine if these results relate to peripheral or central taste system maturation, recordings were made from the chorda tympani nerve in 8 sheep calf aged 108-110 days of gestation (term=147 days) and 4 lambs aged -50 days after birth. Both whole nerve and few or single fiber (n=13) responses were recorded while stimulating with the 0.5M NH₄Cl, KCl, NaCl and LiCl. The whole nerve responses were passed through an AC to DC converter, and the frequency of single and few fiber responses was counted using a rate meter. Effectiveness of chemical stimulation was then evaluated on the basis of height of the whole nerve response, or frequency of unit responses.

Analysis of both whole nerve and unit data demonstrated that in 108-110 day fetuses the most effective stimuli are NH₄Cl and KCl, that is, in that order. Although responses were recorded to lingual stimulation with NaCl and LiCl, they were minimal. In the lamb, NH₄Cl remains the most effective stimulus; however, compared to the fetal effective and KeCl LiCl become much more effective. Thus, the preliminary data indicate that the order of effectiveness of the four salts is: Fetus: NH₄Cl > KCl > NaCl = LiCl. Lamb: NH₄Cl > KeCl > NaCl = LiCl.

The mean response frequency for all four chemicals increases with age. Averaged frequency for NH₄Cl in the fetus is 47 impulses/sec while the frequency in the lamb is 82 impulses/sec, an increase of 74%. The change in frequency for KCl is much less, however, the increases for NaCl and LiCl are very striking, at 248% and 284%, respectively.

These changes in peripheral taste nerve function during development confirm the reported central nervous system changes. At both levels, NaCl and LiCl become more effective stimuli as development progresses. The results support our hypothesis that the developmental change in salt taste responses is related to receptor maturation rather than maturation of taste synapses and fiber tracts. (Supported by NSF Grant BNS 77-09920 and National Institute of Dental Research, N.I.M., Research Career Dev. Award DE00069 to C. M. M.)

CHEMICAL SENSES: SMELL AND TAST

Previous behavioral studies in zinc deficient rats(Zn-) demonstrated abnormal preferences for neutral chloride (NaCl), sodium (Na), quinine (Q), and hydrochloric acid (H) solutions when tested in a 2-bottle preference procedure(J.Nutr. 109:436-442, 1977). We hypothesized that zinc deficiency would result in a decrease in taste sensitivity and therefore measured the integrated whole nerve chorda tympani responsiveness to taste solutions in control and Zn- rat strains. The test rats were male rats initially weighing 125-150 grams; they were fed either a Zn- diet (1.3 ppm Zn) ad libitum or pair fed a Zn+ diet (100 ppm Zn) for 10 weeks. These rats were tested for moderate symptoms to develop including anosmia, failure to grow, alopecia, and mild inflammation and scaling of the paws; control rats appeared normal.

The right chorda tympani was dissected free using a microsurgical approach, sectioned centrally, and the proximal end placed on a nichrome wire electrode. Response of the whole nerve was differentially amplified and fed into a Behler sumator and ink writer; time constants were 0.6 seconds (s) rise and 2.6 s fall. The rats anterior tongue was enclosed in a flow chamber fitted with a rubber dam to exclude saliva. Solutions were delivered by gravity flow for 15 s and always immediately preceded and followed by 15 s of water flow. A series of 10 N (0.00003 to 1.0 M), 5 S (0.01 to 1.0 M), 7 G and 7 H (0.00001 to 0.01 M) solutions were utilized. Peak amplitude of the charted record was used as an indicator of total neural activity.

To assess the initial statistical analysis of the frequency of responses above and below the median response magnitude of both groups for rats of each concentration in the series; chi-square analysis of the data indicated that responses were significantly (p<0.001) fewer responses above the median and more responses below the median, as compared to the Zn+ rats. In addition, the data indicated utilization of different coordinate systems frequently used in electrophysiological research such as Response(R) vs. log concentration(C), log R vs. log C, 1/R vs. 1/C, and R vs. C/R. Results also demonstrated significant differences between the responsiveness of the groups.

This study suggests that the integrated whole nerve chorda tympani responsiveness to taste solutions flowed over the tongue of zinc deficient rats is significantly decreased compared to control animals. These findings support and extend the results of previous behavioral studies and the etiology or locus of the defect is unclear. Further research is indicated.

Temporal characterististics of responses by single neurons of the rat NTS to 50 widely different stimuli were compared, and their similarities computed by comparing the direction and amount of change in firing rate from one 0.1 sec interval to the next for 2 sec. The overall similarity value was the algebraic sum of the largest difference in the 0.1 sec interval of the stimulus. Similarity values for replications of the same stimulus across many different neurons were significantly higher than those for different stimuli, even within the same neuron.

To determine whether it is possible to identify a stimulus solely on the basis of its temporal response pattern, multiple responses to 4 different stimuli (NaCl, HCl, Quinine HCl, and sucrose) were averaged, giving “standard responses” for each stimulus. Individual “test responses” to the 4 stimuli were compared to the standards, and the standard giving the highest similarity value was taken to be the predicted stimulus identity for the test response. Identification was correct at least 70% of the time, and this was true over a fairly wide range of test response concentrations. If the threshold for this test was increased to 25%, test stimuli were still most often identified with the correct standard, and errors in identification usually involved confusion with stimuli known to be similar in taste(e.g. NaCl might be identified first as another Na or Li salt, but showed very low similarity). These results strongly suggest that temporal aspects of the neural response make an important contribution to the overall taste quality in rat taste, and electrical stimulation studies to demonstrate whether these temporal patterns are actually used by the animal to discriminate stimulus quality are presently underway.

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OLFACTORY BULB NEURONS TERMINATE IN THE RECEPTOR EPITHELIUM. Roger E. Davis, Neurosciences Laboratory, The University of Michigan, Ann Arbor, MI 48109.

A radioautographic investigation of the fiber projections of the olfactory bulb of the teleost Macropodus opercularis revealed that bulbar neurons enter the olfactory nerve and terminate selectively in the olfactory receptor epithelium. Four adult Macropodus were administered 0.2 to 0.5 µCi of [³H]-2,3]proline on a dry 60 to 80 µm diameter head of Dow resin which was implanted unilaterally in the olfactory bulb (Davis, R.E. and Agrawon, B.W., 1977, Brain Res. 126:341-346). Following a 10 day survival period, the head was removed, fixed in formalin, decalcified and embedded in paraffin. Ten micron horizontal or transverse sections were dipped in Kodak NBT-2 emulsion and stored for 3 weeks. Following development of the emulsion the tissue was lightly stained with cresyl violet acetate. The implanted bulb was heavily labeled by grains of reduced silver. Labeled protein was distributed in the ipsilateral olfactory nerve and olfactory receptor epithilium in the nasal sac. The segments of indifferent epithelium were lightly labeled. The contralateral olfactory bulb and nerve were also only lightly labeled. The pattern of labeling in the nerve suggested that axons of efferent neurons intermingle with the afferent primary receptor neurons. Similar results have been obtained in the goldfish, Carassius auratus.

The cells of origin of efferent fibers in the olfactory nerve were investigated using the peroxidase method (Coleman, D.K., Scalil, F. and Cabral, E. 1976, Brain Res. 102:156). A 1 x 2 mm piece of filter paper was soaked in saline containing 0.4 µg horseradish peroxidase per 10µl and placed in the nasal sac for 40 hours. Carassius was used in this experiment. The brain, olfactory nerves and epithelium were embedded in gelatin to obtain 40 micron thick horizontal sections. The diaminobenzidine tetrahydrochloride procedure was used to localize the peroxidase reaction product in unstained sections. Control sections were stained with cresyl violet acetate.

The olfactory bulb on the side which received the HRP and the contralateral bulb showed similar distributions of endogenous peroxide in capillary walls and erythrocytes. Fibers in the superficial layer of the anterior zone of the ipsilateral bulb contained reaction product. The cells of origin of efferent fibers in the olfactory nerve were also labeled. The data indicate that the olfactory efferent neurons in fish terminate selectively in the olfactory receptor epithelium, which suggest a receptor modulation function. However, whether the afferents synapse with receptors or some other cells remains to be investigated.

DIFFUSION OF TASTE STIMULI TO FUNGIFORM TASTE BUDS IN ZINC DEFICIENCY. John E. eBook and Lloyd M. Heidel, Department of Biological Science, Florida State University, Tallahassee, FL 32306.

Zinc deficiency (zn-) produces altered taste sensations in humans (elevated detection and recognition thresholds) and altered taste preference behavior in rats. The exact mechanisms responsible for these effects are not known. Several laboratories have shown that zn- produces a hyperkeratosis of the tongue's dorsal surface in a variety of species. In our laboratory this hyperkeratosis accompanying zn- has been seen to completely cover fungiform taste buds or to gradually build up and surround fungiform papillae which resulted in "pit-like" structures with taste buds submerged 100-200µ below the epithelial surface. The present study was undertaken to determine the diffusion of taste stimuli to taste cells in control and zn- rats. The rates of diffusion of various stimuli (representing the 4 taste qualities) were computed as a function of time of application and distance from the taste pore to the tongue's dorsal surface. These calculations show that the increased distance to the taste cells in zn- rats dramatically reduced the rate of stimulus concentration increase at the taste cells. These results suggest that altered taste sensation in zn- and tasting preference behavior in zn- animals may be explained by the decreased rate of taste stimulus onset and concentration at the taste pore as calculated by diffusion processes.

For stimulation, we utilized a distinctive pattern of 2-deoxyglucose (2DG) uptake in the glom ular layer of the rat olfactory bulb, as detected by the Sokoloff autoradiographic method. The results suggested increased ability, over that of water itself, to sorb and retain certain types of odorant molecules. (NIH Grant NS 03904)

We have sought further information about the contribution of receptor axons to the patterns by examining 2DG uptake associated with selective electrical stimulation of the olfactory nerve. The results were unexpected in that stimulation through a bipolar electrode while recording evoked potentials in the bulb. Stimulation of the nerve bundles in the dorsal recess of the nasal cavity produced evoked potentials limited to the lateral anterior aspect of the ipsilateral bulb. Autoradiography revealed an intense focus of 2DG uptake in that region. Low frequencies (1/4 sec.) and low intensity of stimulation did not produce patterns characteristic of the CT responses. This suggests that for butanol, uptake by olfactory mucosa is not significantly different from uptake by water. Therefore, the air/water partition coefficient was in good agreement with that reported by Amoore (Fulmarus glacialis). This agreement supports the validity of the present technique. We determined that, after equal exposure times, the mucosal uptake was about 1.5 times that of water, indicating that odor induced 2DG uptake is primarily due to increased activity in olfactory axons.

Similar experiments have been conducted in the isolated turtle olfactory bulb. The Sokoloff method was adapted to the in vitro preparation by continuously infusing the bath with 14C-2DG (50µl) during the 45 min. period of stimulation. This was followed by a 15 min. washout with turtle Ringer prior to freezing and staining preparation for autoradiography. Stimulation was maintained in intensity and delivered at a rate of 1/2-2 sec. while recording evoked responses in the bulb. Stimulation of an entire nerve bundle was utilized. No significant differences were found between the rise responses in the bulb produced extensive 2DG uptake in the corresponding part of the bulb. Following these relatively intense volleys, the induced 2DG uptake was seen throughout most of the bulb. This result is similar to the uptake seen in vivo, and we conclude that the Sokoloff method can be applied successfully to in vitro preparations of the CNS, and moreover that the results are qualitatively similar to those observed in vivo.
MECHANOSENSORY INFORMATION BY APLYSIA NEURONS. Behrus Jahan-Parwar and Steven M. Fredman. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have examined the responses of bilateral suction ablation of the GNC or sham surgery. For aversion conditioning. In this experiment we tested the role of the cortex (GNC) has been implicated in both neophobia and taste aversion training rats were first accustomed to a restricted drinking site. Odor and taste in compound also evoke a strong neophobic response before conditioning. The gustatory neophobic responsiveness of the B neurons to the odor/taste compound that was found in the sham controls, 2) odorants exhibited anterior patterns (limonene, camphor, pinene, and vanillin; B-hexachloroethane); and 3) appeared to stimulate the epithelium (trimethylamine, cinnamic aldehyde, cantharidin, napthalene, methyl mercaptan, vanillin, B-ionone, acetophenone, valeric acid, and terpineol); 4) odorants exhibited anterior patterns (limonene). The average composite difference in sensitivity expressed as a bell-shaped and suggested that mechanisms of stimulation/inhibition by the amphipathic molecules, KGF and ZjE-A, with stimulation/inhibition by the amphipathic fatty acid salts (Dethier and Hanson, 1983) support the notion (Dote et al., 1974; Kennedy, 1977) of a role for surface active properties in the action of gymnemic acids and ziziphin. The average composite difference in sensitivity exceeded one order of magnitude.

Experiment 2. We mapped EOG responses to odorants on 17 ventral and 6 dorsal surfaces. Eighteen to 25 positions were selected on each surface and a fixed stimulation sequence was constructed. Each odorant was presented at one concentration (usually one that produced 1.5 mv EOGs in previous experiments) sequentially to each point on the epithelium. Each receptor cell responded to one or more than one odorants. For each mucosal surface and at all concentrations the anterior epicrine position was more sensitive to butanol while the posterior position had maximal responses to sucrose. The average composite difference in sensitivity exceeded one order of magnitude.


In normal rats, odor alone is a weak conditioned stimulus (CS) for LiCl-induced illness (US); taste alone is an effective CS. However, if odor and taste are presented as a compound CS and following by lithium chloride, both odor and taste become effective CSs when tested alone. Odor and taste in compound also evoke a strong neophobic response before conditioning. The gustatory neophobic responsiveness of the B neurons to the odor/taste compound that was found in the sham controls, 2) odorants exhibited anterior patterns (limonene, camphor, pinene, and vanillin; B-hexachloroethane); and 3) appeared to stimulate the epithelium (trimethylamine, cinnamic aldehyde, cantharidin, napthalene, methyl mercaptan, vanillin, B-ionone, acetophenone, valeric acid, and terpineol); 4) odorants exhibited anterior patterns (limonene). The average composite difference in sensitivity expressed as a bell-shaped and suggested that mechanisms of stimulation/inhibition by the amphipathic molecules, KGF and ZjE-A, with stimulation/inhibition by the amphipathic fatty acid salts (Dethier and Hanson, 1983) support the notion (Dote et al., 1974; Kennedy, 1977) of a role for surface active properties in the action of gymnemic acids and ziziphin. The average composite difference in sensitivity exceeded one order of magnitude.


Gymnemic acids and ziziphin (from Gymnema sylvestre and Ziziphus jujuba) suppress fly behavioral and neural responses to a variety of odorants in a manner that in which they suppress sweetness perception in humans (Kennedy et al., 1975; Kennedy and Halpern, 1977). The lack of a monosaccharide isotherms in compounds from G. sylvestre (KGF) (Bartoshuk et al., 1969) and Z. jujuba (ZjE-A) (Kennedy, 1977) stimulated two cells exhibiting taste and olfactory responses to alcohols. Responses to KGE and ZjE-A presented either as single solute stimulants, in mixtures with sucrose, NaCl, or 50mM LiCl, or during mechanical stimulation, suggested that the two types of responses that were not the "auger" of meoneuro-receptor cells. Responses to KGE, ZjE-A, 50mM LiCl, NaCl, or sugars after adaptation to sucrose, KF, or distilled water, confirmed that these solutions to KGE and ZjE-A stimulate the "water" and "fifth" cells. Responses of the fifth cell to 2 sec stimulations with KGE and ZjE-A were concentration dependent, but slow and not always phasic-tonic. Prolonged stimulation (10 min) often led to volleys and cessation of firing. A 1 min pretreatment with KGF or ZjE-A resulted in an initial depression, subsequent recovery, and eventual increase and volleys of action potential responses to all odorants.

Dose-response curves for stimulation of the "fifth" cell and inhibition of the response to sucrose were bell-shaped and suggested that mechanisms of stimulation and inhibition are related. Consequently, it is unlikely that KGF and ZjE-A suppress sucrose perception by "occupying" and thus reducing the receptor sites. The stimulation/inhibition effects suggest disruption of the plasma membrane. Similarities of stimulation/inhibition of cell responses to KGE and ZjE-A, with stimulation/inhibition by amphipathic fatty acid salts (Dethier and Hanson, 1983) support the notion (Dote et al., 1974; Kennedy, 1977) of a role for surface active properties in the action of gymnemic acids and ziziphin.
At low stimulus concentrations type I responses are characterized by an increase in activity within one second of stimulus onset. The activity of type I neurons is suppressed during the stimulus event when these units were stimulated with 2 second odorant pulses delivered by an air dilution olfactometer and their responses were quantified as PSTH data obtained from units driven by varying concentrations (14c). Increases in activity following stimulus offset (off responses) were apparent for some units and appeared to be of two types: phasic and tonic. Off responses occurred in some cells that did not respond during the stimulus event and in some of the cells exhibiting type I, II, and III responses during the stimulus event. It thus appears that the occurrence of off responses is not dependent on a unit’s response during the stimulus event.

The hypothesis that some volatile compounds can enter the vomeronasal organ during urine investigation was pursued through the use of fluorescent dye-adulterated urines. Guinea pigs were offered urine with or without a fluorescent dye, rhodamine B or 6-carboxyfluorescein (6CF). A short exposure to the urine (less than 10 min), the guinea pig was sacrificed and the vomeronasal organ was removed, frozen, sectioned, and examined with a fluorescence microscope. In animals exposed to unadulterated urine, no fluorescent signal was observed in tissues from animals exposed to unadulterated urine. Additionally, if the vomeronasal system is utilized in the detection of female stimuli, then activation of the vomeronasal epithelium should be reflected as an increase in activity of central nervous system structures associated with the vomeronasal system, e.g., the accessory olfactory bulb. For guinea pigs exposed to 2 second odorant pulses delivered by an air dilution olfactometer and their responses were quantified as PSTH data obtained from units driven by varying concentrations (14c). Increases in activity following stimulus offset (off response) were apparent for some units and appeared to be of two types: phasic and tonic. Off responses occurred in some cells that did not respond during the stimulus event and in some of the cells exhibiting type I, II, and III responses during the stimulus event. It thus appears that the occurrence of off responses is not dependent on a unit’s response during the stimulus event.

Although the projection from the individual PSTH data obtained from units driven by varying concentrations (14c). Increases in activity following stimulus offset (off responses) were apparent for some units and appeared to be of two types: phasic and tonic. Off responses occurred in some cells that did not respond during the stimulus event and in some of the cells exhibiting type I, II, and III responses during the stimulus event. It thus appears that the occurrence of off responses is not dependent on a unit’s response during the stimulus event.

The hypothesis that urine investigation activates central structures in the olfactory and accessory olfactory pathways was investigated using the 1H 2-deoxy-2-glucose (2DG) technique. Male guinea pigs were injected with 2DG and exposed to a series of female urine samples during the 2DG incorporation period. Autoradiograms revealed activation of the vomeronasal organ and the accessory olfactory bulb after exposure to female urine. To our knowledge, this is the first demonstration of activation of the vomeronasal organ by stimulation of female urine. These results suggest an active response of the vomeronasal organ by liquid borne, possibly high molecular weight, compounds.

Bipolar electrical stimulation of either the lateral olfactory tract (LOT) or olfactory bulb (OB) in urethane anesthetized rats (1.5g/kg) male rats (300 grams) elicited characteristic field potential and single unit responses in the ipsilateral piriform cortex. An air-dried glycerin-microsphere was delivered to dorsal or ventral surfaces of the caudate nucleus. Pulses of 1-carvone to cells identified with electrical stimulation. A tracheal tube and a tube ascending to the caudate nucleus were inserted and connected to a Harvard Instruments syringe. Sodium citrate (1.6M) filled micropipettes (20 MΩ) were inserted from dorsal to ventral through the rats’ cortices. Neuronal recordings were made in both anterior (3-4 mm anterior bregma) and posterior (at bregma) piriform cortex.

A total of 38 cells were studied in response to electrical stimulation. LOT stimulation was used to elicit responses in 9 cells which showed latencies to spike initiation ranging from 3 to 16 msec. with an average of 9.1 msec. OB stimulation was used to elicit responses in 29 cells with latencies to spike initiation ranging from 4 to 28 msec. with an average of 13.3 msec. 10 of these 38 cells were studied intracellularly and exhibited latencies of 1-6 msec. to initiation of the EPSP. 28 of the total 38 cells were subjected to odor stimulation at 1-carvone (1 part/100). In 22 extracellularly recorded cells, 11 were excited and 11 showed no response to odor stimulation. In 6 intracellularly recorded cells, all showed excitatory responses although some showed no action potentials in response to odor stimulation, but RFSFs were present. Latencies to spike initiation ranged from 1.1 to 5.0 sec. in response to odor stimulation.

These results indicate that piriform cortex neurons respond to 1-carvone stimulation with excitation. Neuronal inhibition was never observed either extracellularly or intracellularly using our stimulation parameters. At this time we can see no difference between the response patterns in anterior and posterior piriform cortex extending our studies using a variety of odors and odor concentrations.

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ACROSS FIBER SPECTRUM FOR SUGAR IN TASTE. Elizabeth Oand and Zacharia, Temple University Health Sciences Center, Dept. of Physiology & Biophysics, Philadelphia, PA 19140.

Diptera chemoreceptors respond to more than one chemical class. Amino acids as a group stimulate two cells and initiate distinct behaviors. We report here that certain sugars may likewise stimulate the same two distinct receptor types.

Using standard tip recording through third molar LCI six sugars were tested over a wide range of concentrations on "largest" labellar hairs (1-12) of intact male Phormia regina, Meigen, 1-3 days old, under a 12/12 light/dark cycle. The occurrence of a significant population of fibers (Smith, Travers & Van Buskirk, 1979; Travers & Smith, 1980) which failed to decline in January through April seems not to be related to the age or sex of the animal. Gerbils with stable and declining taste responses had identical age ranges (12 to 36 weeks) and no significant difference in mean age (p > .15, chi square). 37% of those nerves giving stable responses were from male gerbils while only 40% of the nerves with declining responses were from male gerbils (p < .15, chi square). We conclude that the physiological taste responses of the gerbil display seasonal variation which may be related to neurotrophic maintenance in the gustatory system.

Supported in part by NS 07012.


Following transection of the gerbil's (Meriones unguiculatus) IXth nerve, summed impulse discharges to taste solutions declined by 50% in 119 ± 44 min (± S.D., N = 15). Compound action potentials were still normal, indicating that the taste tract remained viable. We noted, however, that the taste responses of a number of the animals remained stable after nerve transection. We defined a stable response as one which was maintained longer than 3.5 hours (the mean decline time plus two S.D.); in fact, most stable responses showed no sign of declining in a 5 hour recording session. Over a four year period we have analyzed the influence of season of the year, age and sex on the likelihood that transection would produce a rapid decline in the ner ve's taste response. 119 IXth nerves were transected in the months September through April. Taste responses declined in 98% (39 of 40) of the nerves recorded from September through December, yet declined in only 73% (38 of 79) of cut nerves recorded from January through April (p < .01, chi square). Gerbils were caged with littersmates of the same sex and maintained on a 12/12 light/dark cycle. The occurrence of a significant population of nerves which failed to decline in January through April seems not to be related to the age or sex of the animal. Gerbils with stable and declining taste responses had identical age ranges (12 to 36 weeks) and no significant difference in mean age (p > .15, chi square). We conclude that the physiological taste responses of the gerbil display seasonal variation which may be related to neurotrophic maintenance in the gustatory system.

CHEMICAL SENSES: SMELL AND TASTE
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TASTE RESPONSES TO L-AMINO ACIDS IN RAT: A SINGLE NEURON ANALYSIS.

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The physiological importance of l-amino acids (aa's) to animals implies that the gustatory system should be sensitive to their presence in the environment. Studies of bacterial chemotaxis and teloestast responses confirm that chemical sensitivity is not unique to aa's in these species is independent of the general systems for processing odors. Afferents to the main olfactory bulb have wide range of taste experience from aa's, with a flat-bitter component predominating for many. The amplitude of whole-nerv chorda tympani responses from rats correlates well with the main qualitative estimates of aa concentration, suggesting that human and rat sensitivities to these stimuli are similar. We sought to further analyze the rat's neural responses by recording the activity of single axons from the chorda tympani. Stimuli were 12 l-amino acids which were washed over the anterior half of the tongue at concentrations which evoked one-half the maximum whole nerve response for that chemical. The mean activity of 40 axons indicated that stimuli had the following order of effectiveness: 0.06 M CTX > 0.3 M ARG > 0.3 M LYS > 1.0 M GEL > 1.15 M PRO > 0.01 M HIS > 0.2 M GLU > 0.2 M LYS. This ranking correlated .07 with that established by whole nerve recordings. Most axons appeared to respond to this range of stimuli with a similar profile, with the overall responsiveness of the cell being the critical variable in determining breadth of sensitivity. For example, if a neuron's response to a standard solution (.1 M NaCl) was robust (.20 spikes/sec), then that cell was likely to respond to some degree to all 12 stimuli. Moderate responses (10 - 20 spikes/sec) to the standard indicated sensitivity restricted to more effective aa's, and weak salt responses accurately predicted activity only to CTX, ARG and LYS. Thus for these stimuli there was basically one neuron "type" with varying degrees of sensitivity to stimuli in general. The relative similarity of any two aa's, as indicated by correlations among across-fiber patterns, was in general accord with that seen in human and rat psychophysical studies. (Supported by NIH grant NS 10405)

EFFECT OF LESIONS ON AMINO ACID DISTRIBUTION IN RAT OLFACTORY BULB


Distributions of GABA, glutamate and aspartate were determined in the olfactory bulb of control and lesioned rats using quantitative histochemical mapping procedures. Data from these rats concerning the choroid plexus of the lateral angles of the third ventricle and portions of the olfactory bulb and olfactory tubercle have been reported previously (NS Abst. 4:86,91, 1978). In rats A and B, knife cuts were placed more caudal to the anterior olfactory nucleus through the LOT, extending more deeply in B than A. In rats C and D, lesions were caudal to the anterior olfactory nucleus through the LOT, extending more deeply in C than D. The significant decrease in glutamate in all layers deep to the glomerular layer in rats C and D is consistent with a centrifugal contribution from olfactory bulb to the superficial layers. (Am. Cancer Soc. BS45; USPHS NS-08862 and NS-08000).

THALAMO-CORTICAL MECHANISMS IN ODOR GUIDED BEHAVIOR: II. EFFECTS OF LESIONS OF THE MDOUDOUSS NUCLEUS AND FRONTAL CORTEX ON ODOR PREFERENCES AND SEXUAL BEHAVIOR IN HAMSTERS.


Hamsters crucially depend on the patency of the main and accessory olfactory systems for successful reproductive behavior. Only recently have modern techniques clarified the secondary olfactory projections and little is known about their functions. This study is an attempt to discover the role of one of the secondary olfactory pathways, the midodoural thalamic nucleus (MD) and associated prefrontal cortex, in natural odor preferences and sexual behavior in hamsters.

Preoperatively, male hamsters demonstrated normal preferences to odors produced in the home cage and normal sexual performance. In two separate experiments, subjects received either sham surgery or lesions limited to MD or to the frontal neocortex either alone or combined with MD. Postoperative odor thresholds of all animals were normal. However, both attraction to pure odors and odors of male and female conspecifics and discrimination among these odors was significantly reduced in hamsters who had received damage to MD and RS but not MW or controls.

In post-operative tests of sexual competence all subjects continued to mate successfully. However, male hamsters who receive lesions of MD or (CD) to the frontal cortex) spent more time sniffing the female's body rather than its genital and often mounted an inappropriate body position of the female.

Only the medial subdivision of MD receives direct olfactory input and only this subdivision projects primarily to RS. It appears that damage to this subdivision could affect autonomic pathways disrupting discriminatory aspects of odor preference and sexual performance, but does not eliminate detection of odors or their potential for "priming" sexual behavior.

MORPHINE INHIBITION OF PARABRACHIAL TASTE UNITS REVERSED BY NALOXONE.

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Immunohistochemical studies have localized endogenous opioid peptide activity within central nuclei (eg. nucleus tractus solitarius) and parabraclial nucleus, and the present data indicate that administration of morphine or its antagonist, naloxone, markedly reduced water intake in water-deprived animals (Rogers, et al 1978, N.Pharmac.Soc.).

Our most recent data indicate that responsiveness of parabrahcal (PBN) gustatory units to stimulation (salt) can be modified by intravenous administration of morphine. These effects on unit activity are readily reversed following naloxone infusion, thus suggesting a specific effect of morphine on these cells.

Rats were anesthetized with urethane (1.5mg/kg) and provided with vena cava cannula. The animals were then mounted into the stereotactic frame; the skull trephinated and glass microelectrodes (filled with Pontamine dye) lowered into the PBN. Single unit responses were monitored with an oscilloscope coupled to an audio-amplifier and a pulse integrator-polygraphic chart recorder. Gustatory units were identified by increased activity in response to washing the tongue with 1.5N NaCl solution followed by a return to baseline activity in response to washing the tongue with water. Upon localization of such a taste activated unit, morphine was infused via the vena cava. Within 15sec, both baseline activity and responses to stimulation were greatly diminished. Although no further decline in baseline activity was seen, responses to stimulation were lost 20min post-infusion. At this time, naloxone was infused via the vena cava and both baseline activity and responses to stimulation were readily returned to pre-morphine levels. The location of such identified cells was then marked by iontophoretic application of Pontamine dye. The responsive cells were thus identified following preparation for standard Nissl histology. Perhaps the suppressant effects on feeding or drinking that are observed with systemic administration of morphine or naloxone are attributable to the activity of these and similar units i.e. "blunting" of taste reception, which has previously been demonstrated to modulate consummatory behavior (Errits & Gorbit, 1973, JCPF).
in the concept of four primaries. However four
inhibitors, there has been no report of marker agents
or inhibitors which preferentially bind to specific
classes of olfactory receptor sites. We are attempting
to develop such agents from a novel source.

Chemical agents once used as war gases (mainly of
pre-World War II vintage) are being tested as possible
probes using the frog nose as a model system. For
example, ethyl bromocacetate (a "fruity-smelling" tear
gas also containing a strong "fishy-smelling" amine,
as well as other "fruity-smelling" esters, but does
not block responses to isovaleryl amine and other "fishy-
smelling" amines. These blocking agents evoked one-half the maximum whole chorda tympani nerve response
for that chemical (see Pritchard and Scott, this volume). In ad-
dition to the agent application, unlike the application of in-
hibitors in liquid solutions which block testing.
CHEMICAL SENSES: SMELL AND TASTE


Until a careful olfactory nerve section was performed on the salamander, Ambystoma tigrinum. Physiological recordings and morphological observations were made at several time points following anotomy to investigate the structural and functional correlations of neural degeneration and renewal in the olfactory epithelium. Slow, transmepithelial voltage-transients (Voeg(-) and Veog(+)), evoked by odor stimulation decreased in amplitude and disappeared within 10 days. Subsequently, they recovered to 80-100% of the amplitude of the contralateral controls by 100 days following nerve section. Unitary activity was virtually absent 10 days following the lesion, but reappeared within 24-60 days. Analysis of the response properties of newly differentiated olfactory receptor neurons indicated similar odor specificities, latencies, intensity-response functions, and adaptive properties as those observed in control units. Light microscopic examination revealed necrosis and loss of the olfactory receptor cell population by 10 days following nerve section, followed by renewal of the neurons and return of epithelial thickness and apparent nuclear density, as compared with contralateral controls, by 30 days. Specifi- cation and chemical staining of secretory products in the apical regions of sustentacular cells suggested no change in the activity or gross structure of these cells throughout the neurogenic process. Physiological activity in the epithelium was observed before macroscopic observation of reconnection of the olfactory nerve to the olfactory bulb and functional activity of terminal synapses in the olfactory bulb is not necessary for transduction and action potential production in these neurons. This study also suggests that the presence of olfactory receptor neurons is necessary for maintenance of both components, Voeg(-) and Veog(+), of the slow voltage-transient response, and that completely functional, newly differentiated neurons are produced in the olfactory epithelium following olfactory nerve section.

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432 EFFECTS OF INTRANASAL ZnSO4 IRRIGATION ON OLFACTORY BULB MORPHOLOGY AND BEHAVIOR IN THE RAT PUP. Pauline Singh, Pat Stadis, Pat Niles, George Pappas, and A. Marie Tucker. All in Einstein Col. of Med., Bronx, NY and Queens Col., Flushing, NY.

Morphological changes in the glomerular layer of the main olfactory bulb and latency to suckling were investigated after a chemical lesion of the olfactory neurons. Wistar rat pups from 7 litters were subjected to intranasal irrigation with 5% ZnSO4, and 7 pups at 7 or 10 days of age. Within 10 days following each ZnSO4-treated (Zn) and 3 normal or saline controls. Body weights were taken daily, and latencies to suckling on the anesthetized mother were observed. Pups were killed at 1, 4, or 7 days post treatment. The olfactory bulbs of both control and experimental animals were then prepared for light and electron microscopic study.

Light microscopy indicated the following changes in various days post treatment: 1 day, discrete fibers were visible in the medial glomeruli, but most of the periglomerular glomeruli appeared disorganized; 4 days, no distinct glomeruli were visible indicating that degeneration had occurred; 7 days, medial glomeruli were again present, although no discrete fibers were visible. This may indicate early stages of regeneration. Electron microscopy showed degenerating terminals with distorted synaptic vesicles, decrease in neuronal density at 1 day post treatment. Some glial-englundged material and membranous whorls were also present. At 4 days post treatment, few or no degenerating terminals were distinguishable. Glial- englundged material was present suggesting that the degenerating terminals had been phagocytosed.

The weights of Zn pups were significantly lower than those of control littermates on all days following treatment (p<.05). The Zn pups killed at 1 and 4 days post treatment exhibited a deficiency in nipple orientation and attachment. Their median latencies to such events were 80 sec. (0-240 sec). For control littermates, whereas for control littermates they were 45 sec. or less (p<.05). At seven days post treatment, there was no significant difference in behavior between control and experimental animals.

Olfactory deafferentation appear to be correlated with the morphological changes observed at the light and electron micro­scopic levels.


Recent studies have provided behavioral evidence that olfaction is the dominant sensory modality for nipple localization by suckling rat pups (Teicher and Blass, Science 193:422, 1976). These studies have implied that the nipples are coated with a lipid-soluble substance that may elicit the suckling response in the pups. As part of a study of the functional development of the olfactory system, we have extended the 2-deoxyglucose (2DG) method of Sokoloff to neonatal animals in order to detect sites for further define the specific sites correlated with pheromone-induced activity in newborn pups.

Supported by NIH grant RR-00165 to the Yerkes Regional Primate Research Center, Atlanta, GA and Grant RR-00165 to the Yerkes Regional Primate Research Center and Yerkes Regional Primate Research Ctr. and Dept. of Anatomy, Emory University, Atlanta, Georgia 30322.

Numerous myelinated neurons and dendritic segments were found in the olfactory bulbs (OB) of 5 normal squirrel monkey (Saimiri) brains prepared for electron microscopy by routine procedures. Morphological profiles occurred in the periglomerular region (PGR), but a considerable number were also present in the external plexiform layer (EPL), especially in its uppermost region. The granule cell layer (GCL) in the OB of squirrel monkeys was established by Pinching and Powell (J. Cell Sci. 9, 171) in rat OB for the classification of different cell types were employed. Although not every cell could be classified individually it was possible to discriminate between 4 different types of myelinated neurons. In the PGR and the EPL, myelinated somata resembled external granule cells, and this cell type was the most numerous of all myelinated cells. Neurons with the characteristic appearance of short axon and medium to small tufted cells were also found. These cell types occurred in about equal numbers. The few myelinated perikarya in the GCL were morphologically indistinguishable from adjacent granule cells except for the myelin sheath. Profiles of myelinated dendritic segments were sectioned in both the transverse and parasagittal plane, and it was evident that they were not running in a preferred direction. Some myelinated dendritic segments were in continuity with somata that resembled tufted or external granule cells. The myelin sheath either completely enveloped a perikaryon or was interrupted by myelin-free gaps in the plane of the section. The myelin also varied in thickness, the thin sheaths exhibiting about 8-12 dense lines. Dendrites also displayed gaps in myelinulation. Frequently, at these gaps, the dendritic segment was engaged in a synaptic relation­ship and was either in a pre- or postsynaptic position. Occasionally, the synapse was reciprocal. The OB of a 44-year-old monkey OB. In the ape, however, myelinated profiles in the OB are less frequent. The functional significance of these myelina­ted profiles in the OB is reflected in recent supported National Institute of Health Grant RR-00165 to the Yerkes Regional Primate Research Center and NIH Grant ET-00638.

434 MYELINATED NEURONS AND DENDRITES IN THE OLFACTORY BULB OF PRIMATES. Margaret Tingey and Johannes Tingey. Yerkes Regional Primate Research Ctr. and Dept. of Anatomy, Emory University, Atlanta, GA 30322.

In 12 of 13 animals there was a focus of 2DG uptake in the dorsal part of the main olfactory bulb, at a position just medial to the accessory olfactory bulb. Correlation with the histo­logical sections indicated that in most cases the focus was localized in or near a small group of glomeruli. Scattered foci were also present in medial and lateral parts of the bulb. In control experiments, pups exposed to room air or pure air showed activity in the above regions, but the overall patterns were not as heavily concentrated in the dorsomedial position. Exposure of pups to amyl acetate gave patterns similar though not identi­cal to patterns found in adult rats exposed to this odor (Stewart, Kauer and Shepherd, J. Comp. Neurol., in press). Increased 2DG uptake was also observed in the lateral periglomerular area of rat pups in all experimental conditions. Experiments are in progress to further define the specific sites correlated with pheromone-induced activity in newborn pups.

The bulk of evidence, in the rat, suggests that neocortical amnesia does not produce permanent deficits in regulated ingestive behavior, i.e. sodium appetite, taste aversion learning and taste discriminations. However, it would seem that the absence of the neocortex does eliminate some ingestive function since the neocortical projection area of the gustatory system communicates with each subcortical gustatory nucleus and that the neighboring oral sensory-motor and visceral cortical regions are sites which produce ingestive responses such as salivation, licking, chewing, swallowing and gagging when stimulated electrically and produce deficits in tongue use testability. We have reasoned that by using a more sensitive test to examine ingestive abilities of the decorticate rat, some permanent disruption of function might be revealed. To accomplish this we examined the ability of the decorticate rat to remember where and how it obtained sodium even though it was not sodium deficient at the time of tasting NaCl.

Male Sprague-Dawley rats (n=9) were decorticated by aspiration in 1 or 2 stages and recovered 2-3 weeks before training. Each water deprived rat was trained to bar press for water (V160pF) during the first phase of the experiment and then for .15 M NaCl. Immediately after the last training session of each phase rats were injected with sodium depleting drugs (DOCA and furosemide) and given salt-free food and water; 24 hrs. later bar presses were counted during a 1 hr. extinction session (Krieckhaus & Wolf, 1969). The results indicate that the presence of the neocortex is not necessary for the salt-trained rat to demonstrate need relevant resistance to extinction. Salt-trained rats pressed the bar at a high rate during extinction while water-trained rats did not at all. In addition, a very brief exposure to saline (1 min. during the last two days of water training) causes resistance to extinction (n=2).

In another experiment the same paradigm was used except that rats (n=5) were trained before a one-stage decortication. After 2½ weeks of recovery rats were injected with sodium depleting drugs and tested 24 hrs. later. Pilot tests had shown that decorticates recover bar pressing by this time and that controls would retain the taste-place association for at least 26 days. Results show that removal of the neocortex does not interfere with the rat's capacity to associate the presurgically tasted sodium with a need condition that was never concomitant with the taste experience. In summary, it was demonstrated that subcortical structures are adequate in the acquisition and retention of a sodium taste-place association. (Supported by NIH AM 21397).

A THEORETICAL MODEL OF THE FLY'S CIRCADIAN SYSTEM. Jacob Zabara and Elizabeth Omand. Temple University Health Science Center, Dept. of Physiology and Biophysics, Philadelphia, Pa. 19140.

Although the endogenous (autorhythmic) nature of the circadian system is generally accepted, little is known concerning its possible basis in neuronal circadian oscillators. Data previously reported from our laboratory is analyzed to present the chemoreceptor model as an experimental model of the circadian system of the fly. The observations relating light and feeding conditions on chemoreceptor discharge are summarized graphically and compared to behavioral observations. The circadian clock is described as an endogenous rhythm, possibly genetically determined, composed of an ordered sequence of autorhythmic units summing to a circadian time period. The summary is accomplished by integrating mechanistic elements which are expressed as endocrine, or central excitatory states. The independent neural units (A = A1, ..., An) are represented as derivative functions:

\[ A_i(t) = \frac{d e}{dt} \]

where \( e \) = excitation, and \( z = \) the equivalent synapse of A. Relating this formalism to the actual discharge of an autorhythmic unit:

\[ f \frac{d e}{dt} = K_1 \frac{d(t)}{dt} + K_2 (t) \]

where \( I = \) interspike interval or spike frequency and \( K = \) proportionality constant:

For simplicity we have restricted this formulation to a first order derivative, although higher orders are involved. It is possible to consider the neuro-membrane as simulated by a simple physical model of a variable capacitor (dielectric)-resistance network. Discharge recordings from the chemoreceptor in light and feeding conditions will be presented to illustrate this aspect of the model. The polarized state of the membrane and the condition of its dielectric as well as the channels for sodium and potassium represent critical elements of the circadian system. The average value \( f(t) \) of a time varying excitability factor \( (f) \) giving rise to a circadian rhythm over a time interval \( (T, T') \) is represented by the following:

\[ S_I = \frac{1}{T} \int_0^T f(t) \sum_{i=0}^n f_i(t) (2z) dt \]

This formulation, for instance, represents enhanced excitation in a fasting fly where the locomotor activity increases to "override" the ordinary circadian rhythm. (Supported by NIH NS 12344.)
COMPARATIVE NEUROBIOLOGY

We have previously demonstrated that bilateral neural connections of crayfish visual system at protocerebral level have an important role in the central modulatory process of electroretinographic (ERG) and eye glow area (EGA) rhythmicity (Barrera-Mera et al., 1978). In order to explore the role of that area of the central ganglion (cg) as a probable site of synthesis and/or regulatory releasing of neurosecretions involved in the control of retinal rhythms, we used techniques of retrograde tracing with horseradish peroxidase (HRP) into the central structures. We report here that EGA changes can be divided into a dorsal pallial field and a ventral pallial field. The posterior lateral line nerves and all N. VIII branches were also labeled.


Eight nerve branches supplying the three canals, saccus, and afferents to the lateral line nerves and all branches of N.VIII. Saccular afferents went both ipsilaterally and contralaterally to a separate group of cells within this nucleus. Projections to the cerebellar crest - n. anterior were noteworthy in that utriculus, saccus, and canal afferents went both ipsilaterally and contralaterally. All afferents from the lateral line nerves and all branches of N.VIII went both ipsilaterally and contralaterally to a separate group of cells within this nucleus. Projections to the cerebellar crest - n. anterior were noteworthy in that utriculus, saccus, and canal afferents went both ipsilaterally and contralaterally.

Neuroanatomical and electrophysiological techniques were used to study fibers of the vestibulocochlear (VIII) nerve of pre-metamorphic Rana orbicularis tadpoles. Lesions of selected branches of the anterior and posterior ramus were made after opening the otic capsule; horseradish peroxidase (HRP) was applied to the cut nerve. In some experiments HRP was pressure injected into the nerve in place of lesion techniques. The capsules were then sealed. After 1-4 days survival, the brains were processed for the HRP reaction product. Stained primary afferent fibers could be traced from the site of the HRP application through the ganglia of the VIIIth nerve to extensive projections in the rhombencephalon—from the granule cell layer of the cerebellum to the level of the VIIIth nerve. Fibers as large as 10 μm could be seen entering the brainstem and turning to form the ascending or descending tracts of the VIIIth nerve, or bifurcating to form both. Collaterals from these tracts could be seen branching perpendicularly into the medial zone of the alar plate. The largest fibers tended to course more medially. More of these fibers were observed from the posterior than the anterior rami. Neither decussation of fibers to contralateral regions nor differential specificity of projections from various branches was observed.

Efficient fibers and somata were labeled in these experiments also. Application of HRP to the anterior ramus revealed a nucleus with more than 30 labeled cells ventral to the sulcus limitans, in the region of the motor nucleus of the VIIth nerve. This efficient nucleus extends from the level of the rostral edge of the VIIIth nerve caudally to the posterior root of the VIIIth nerve as far as the caudal tip of the neural canal. It contains the posterior somatic nucleus, a different localization of efficient somata. At the level of the VIIIth nerve the cells were found in a more dorsolateral position relative to the anterior ramus described. One cell was found in the lateral reticular zone as far caudally as the Xth nerve. Additional evidence for the anterior ramus efficient nucleus was obtained by injecting HRP into cells identified by anterograde stimulation. Location of these cells was accomplished by first identifying the maximum extracellular field potential evoked by VIIIth nerve stimulation. The recording side was found where efficient cells could be penetrated. Recovered cells were found in the efficient nucleus, with presumed axons projecting towards the VIIIth nerve.

Research supported by NSF and NIDA.


The existence of an orderly representation of visual information in the midbrain tegmentum of the frog has long been known. More recently an orderly representation of auditory inputs in the underlying torus semicircularis has been described (Pettigrew et al. Nature 222:192, 1969). We are currently investigating an orderly representation of somatosensory information in the frog's midbrain. We here report electrophysiological studies on the organization of cutaneous inputs to the midbrain of Rana pipiens.

Multiunit responses to visual, acoustic, and tactile stimuli were recorded with glass micropipettes filled with a solution of pontamine sky blue or para-phenylenediamine for light microscopy, and with lead citrate and uranyl acetate for electron microscopy. Pigment granules were present in the pigment epithelium layer. Two types of photoreceptor cells were seen. One cell type is typically rod-like with a long cylindrical outer segment and an inner segment of the same diameter as the outer segment. A second cell type has a conically tapering outer segment. Using light microscopy, the nuclei of the two photoreceptor types stain anatomically duplex.

The reptilian anterior dorsal ventricular ridge (aDVR) was traditionally regarded as homologous of the mammalian basal ganglia. However, recent connective, histological, and developmental studies indicate that insectivore may be a homolog of the dactylar zone of the mammalian neocortex which receive ascending thalamocortical sensory projections. To study the projection of the aDVR further, injection of horseradish peroxidase (HRP) into the aDVR of Iguana iguana, processed after deLomos and Heimer (Neuosci. Let., '77) were used to label retrogradely labeled cells.

After large injections of HRP into the aDVR, the distribution of labeled cells corresponded to previous descriptions of projections to the aDVR in other reptiles. Thalamic nuclei with labeled cells included nucleus rotundus, nucleus medialis, and several nuclei surrounding nucleus rotundus, such as the nucleus dorsomedialis anterior and dorsolateral anterior. In addition labeled cells were found in several regions within the lateral mesencephalic tegmentum. Very small, restricted injections of HRP were also made in various regions of the aDVR. After a small injection limited to the middle third of the rostral aDVR, labeled cells were found in the aDVR ventral to the injection site. Several large cells lying close to and within the lateral forebrain bundle were also labeled. A few scattered cells were labeled in the lateral hypothalamus and in the lateral mesencephalic tegmentum. The labeled fibers course predominantly in the lateral forebrain bundle and several labeled fibers were also in the lateral part of the medial forebrain bundle. A small injection limited to the middle third of the caudal aDVR, labeled cells were found in the posterior DVR and in the rostral parts of the anterior and lateral hypothalamus. A few labeled cells were scattered in the lateral hypothalamus and in the lateral mesencephalic tegmentum. Labeled fibers coursed along the lateral aspect of the medial forebrain bundle.

This work was supported by NSF Grant BNS77-26022 to ABB.

Supported by PHS Grant EY-01658 and an Alfred P. Sloan Research Fellowship to PG.
A THALAMIC RELAY NUCLEUS FOR THE LATERAL LINE SYSTEM IN TELEOST FISH. THOMAS E. FINGER. DEPT. ANAT., U. COLO. MED. CTR., DENVER, CO 80262.

The lateral line system in teleosts is a sensory hair-cell system comparable to the auditory and vestibular systems of tetrapods. In fish, the lateral line nerves project to the lateral line lobes in the medulla which in turn give rise to a crossed connection terminating in the torus semicircularis located in the dorsal mesencephalon. However, higher order connections of the lateral line system are largely unknown.

Anterograde transport methods (HRP and tritiated amino acids) were used to determine the projections of the torus semicircularis into the lateral line lobes of chick, goldfish, and mormyrid in two species. J. Comp. Neurol. 1978, 180, 59-72.


The central projections of the two anuran auditory organs, the basilar and amphibian papillae, were examined using horseradish peroxidase (HRP) or a combination of blue and Fast Green FCF. The HRP reaction product was visualized by using a cold light microscope. The reaction product on slides. This modification facilitates tissue examination of pigeon brains prepared for catecholamine fluorescence revealed in the posterodorsal lateral "neostriatum" just under the ventricle, a somewhat poorly delimited region with rich fluorescent innervation forming perineural nets. This region was easily distinguishable from the paleostriatum augmentatum, accumbens, and septum. Additional experiments indicated that this "neostriatal" innervation is dopaminergic rather than noradrenergic. Thus, the posterodorsal lateral "neostriatum" in the pigeon brain may correspond to PF in mammals.


HISTOCHEMICAL STUDY OF THE MONOAMINE CELL GROUPS OF THE AVIAN BRAIN STEM. LORRAINE DUBÉ AND ANDRÉ PARENT (SP/UE: R. BOUCHER). LAB. NEUROBIOLOGIE. FAC. MED., UQTR. LAVAL, QUÉBEC, CANADA.

The distribution of catecholamine (CA) and serotonin (5-HT)-containing neurons in the brain stem of the chick was investigated by various histofluorescence methods. The CA cell groups were also studied with the help of histochemical procedure for the demonstration of acetylcholinesterase (ACHE).

In the mesencephalon of the chick, four CA cell groups are found at the level of the III nerve. First, a few large-sized neurons occur in the periaqueductal gray. These neurons do not display any significant AChE activity. Numerous multipolar cell bodies are also present in the tegmental pedunculo-pontine nucleus (TPP). These neurons stain moderately for AChE. Numerous other CA neurons (without AChE activity) are scattered dorsolaterally to the TPP group. Finally, a dense population of large, multipolar, CA cell bodies occurs along the lateral border of the III nerve root fibers. They display a very high AChE activity. Here caudally, a CA and a 5-HT cell group are intermingled together within the decussation of the cerebellar peduncles. The CA cells are small, round or oval and display a very high AChE activity. The 5-HT neurons are larger, multipolar and also strongly stained for AChE. At the level of the nucleus of the IV nerve, numerous 5-HT cells occur along the midline and dorsal midline adjacent to the nucleus of the V nerve. In the caudal and intermedial to the XIIth nerve, numerous 5-HT cells are found within the torus peduncular nucleus at the level of the inferior olivary nucleus. Finally, two groups of CA cells can be visualized in the medulla oblongata. Packed CA neurons are scattered dorso-laterally to the motor nucleus of the X nerve, in an area containing numerous highly reactive AChE neurons. Second, multipolar CA cells are scattered dorso-laterally to the torus semicircularis.

As a whole, the pattern of organization of MA cell groups in the avian brain stem appears complex and somewhat similar to what has been found in mammals. The 5-HT and the CA neuronal systems, however, are more intimately associated one with another in birds.
COMPARATIVE NEUROBIOLOGY

450 LIVING TOGETHER WITH MINIMAL COORDINATION: COMMUNAL SPIDERS. W. David Hollar* and Peter N. Witt. Mental Health Research, R. C. Department of Human Resources, Raleigh, N. C. 27611.

Still photographs, time lapse movies, and direct observations of a species of communal spiders, Misses gregalis (M. g.) Simon, were used in examining spatial distribution, activity level and interactions under various laboratory conditions. M. g. reside together, communally catching prey and feeding, but exhibiting no intraspecific aggression, for social reasons, natural settings, but its several thousand animals. Though other species (e. g. bees, ants) also live in large groups, M. g. (apparently not a hominid) do so without obvious behavioral based castes, division of labor, or other systematic organization. Moreover, the relative lack of interdependence of individual animals has been indicated by naturalistic ob-


An investigation was made of the ascending spinal degeneration to the dorsal column nuclei (DCN) of a non-limbed reptile. Garter snakes (Thamnophis sirtalis) were used in spinal section at levels ranging from segment 2 to 31 and were main-
tained postoperatively for 11 to 28 days. Following perfusion and fixation the anterograde degeneration from degenerated axons according to the Nauta silver method.

The superficial zone of the dorsal column (DC) contains ascend-
ing degenerated axons while caudal to the lesion the deeper zone shows scattered descending fibers. A comparison of the first cord segment of all snakes shows degeneration in the dorsomedial part of the DC following caudal lesions. More rostral cord lesions produce additional degeneration more laterally in the DC. That portion of the DC adjacent to the dorsal median sulcus contains degeneration from all spinal levels. Caudal to the obex degenerated axons from this sulcal zone course ventrally to the nucleus of Bischoff, a paramedian row of cells. This nucleus appears to receive a somatotopic projection from the cord rostral to segment 31.

The dorsal column nuclei (DCN) consist of a thin lamina of cells medially contiguous with a relatively large population of cells laterally. The ventrolateral nuclear portion is wedge shaped and bordered laterally by tractus and nucleus descendens trigemini. The medial boundary at caudal levels is formed by nucleus and tractus solitarius and more rostrally by nucleus de-

452 THE COURSE OF THE DORSAL LATERAL OLFACTORY TRACT AS AN INDICATOR OF DICHTOMY IN THE PHYLOGENY OF PLACENTAL MAMMALS. John Irwin Johnson, Robert C. Switzer III, and John A. W. Solnick. Neuroscience Program and Zoology, Biophysics and Psychology Depts., Michigan State Univ., E. Lansing MI 48824; Lab. of Brain, Evolution and Behavior, NIMH, Bethesda MD 20014; Still photography, time lapse motion pictures, and behavioral observations were used as an index of social organization (animal distances) were used as an index of social organization (animal distances). Behavioral observations were examined by analyzing films of colonies with and without water present under three conditions: heavy fed, light fed, or dextroamphetamine treated. The presence of water gen-


The full extent of the cortical field of origin (FO) of the anterior commissure (AC) of the rhesus monkey was mapped by horse radish peroxidase (HRP) histochemistry. Following complete cal-

lesions commissurotomy at eight anterior commissure levels, and underdarted a second operation involving the massive unilateral in-

jection of HRP into the entire lateral temporal lobe. Since the AC was the only commissural pathway providing a bilateral projection of cortical neurons to the contralateral hemisphere, the FOs of cortical neurons to the contralateral hemisphere were studied. The FO of cortex in areas 17, 18, and 19 was found to be much more extensive than the terminal projection field of the AC as delineated by previous investigators in rhesus (Zeki, Journal of Comparative Neurology, 142, 1973) and squirrel (Pandya et al., Brain Research, 15, 1973) monkeys. These investigators found the terminal projection field limited to the anterior tem-

cral cortex, if any. The FO extends across the entire hemisphere, then the pyramidal cells appearing in the periphery of the hemi-

sphere. This heterotopic organization may be consistent with Van Alphen's (Acta Physiol. Scand. (Pam) N. 1. C. Colby and in that of Georgewo University. Frome of A. Butler and O. Solnick, in text to own collections.)
Figure 1 illustrates this changes few which were unaffected by the lesions. These included previously classified as S or somatic synapses, and apparently normal numbers of flattened vesicle synapses were also seen to be normal. This may involve the formation of heterologous crest synapses, in Area X, LPO , and paleostriatum augmentatum (PA). The degeneration of endings in the interpeduncular nucleus was the same in both sexes. This case consisted of: 1) occasional axon terminals with osmiophilic ovoid granules which probably correspond to lipochondria (Krauhs et al. Biochem. Biophys. Acta, 471, 25, 1977), are involved in the extraretinal bilateral light adaptation of the visual system of these animals. In the direction of the sensory root of IX suggesting that these afferents are amongst those supported by NIH grants HD 08658 and RR 00169.

The afferents from the dorsal and ventral tegmental nuclei, which form a tegmentopeduncular tract, have been studied by the electron histochemical degeneration method in adult rats. Electrolytic lesions were placed stereotaxically so as to destroy the tegmentopeduncular transect as well as the cell bodies of the sensory fibers in the pneumogastric g. HRP was never found in cell bodies of the vagal (X) component of the pneumogastric g.


Figure 2 illustrates this changes few which were unaffected by the lesions. These included previously classified as S or somatic synapses, and apparently normal numbers of flattened vesicle synapses were also seen to be normal. The present study was accomplished at the fine structural level the afferents from the tegmentum caudal to the interpeduncular nucleus. A part of those synapses previously classified as F or as G synapses, and possibly as F synapses are of this origin. It can be hypothesized that these afferents are amongst those which undergo degeneration following unilateral lesions. This may involve the formation of heterologous crest synapses, the increase in number of somatic synapses, or both. The methodology is under testing in double and triple lesion experiments.

Supported by NIH grants HD 08658 and RR 00169.

Electrolytic lesions were placed stereotaxically so as to destroy the tegmentopeduncular tract as well as the cell bodies of the sensory fibers in the pneumogastric g. HRP was never found in cell bodies of the vagal (X) component of the pneumogastric g.

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Electrolytic lesions were placed stereotaxically so as to destroy the tegmentopeduncular tract as well as the cell bodies of the sensory fibers in the pneumogastric g. HRP was never found in cell bodies of the vagal (X) component of the pneumogastric g.
IDENTIFICATION OF EIGHTH NERVE EFFERENT CELLS IN THE BOWFIN, AMIA CALVA. Catherine A. McCormick and Mark R. Bradford, Jr. Department of Anatomy, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C. 20007.

In mammals and in birds two ascending pathways, the tectofugal and thalamofugal pathways, conduct information from the retina to the telencephalon. In mammals and birds the response properties and receptive field size of visual neurons within these pathways suggest a functional distinction. The cells of the tectofugal pathway have relatively small receptive fields and show varying degrees of preference for the orientation of a stimulus. These cells are presumed to be particularly well suited for processing detailed spatial information. In contrast, the cells of the tectofugal pathway show consistently large receptive fields and a much wider range of receptive field sizes. In general, motion and direction are the preferred stimulus features. These cells appear less well suited for carrying precise spatial information and are presumed to be involved with background events and the location of stimulant within the visual field. Some support for this functional distinction is provided by lesion studies in mammals (rhesus monkey, bushbaby and rat) in which lesions of the chalzomfugal pathway produce amnesia in visual acuity while lesions of the tectofugal pathway do not.

The present study was designed to determine whether this distinction applies to visual system. Visual acuity determinations were made for 19 pigeons trained to discriminate high contrast square-wave gratings of spatial frequencies from a blank stimulus of equal average luminance. The gratings and blanks were presented according to the method of constant stimulus. Video-taped motion pictures of the key-pecking response provided an estimate of the eye to stimulus distance. The preparative results indicated that the mean near-field visual acuity of the 19 pigeons was 2.3'). (Corrected range 1.6 - 3.6). Further we have estimated that the visual sensitivity of the pigeon was 2.9 cd/m².

The above data suggest that the morphology of the clitoris is significantly different from that of the penis and thus the two are not truly homologous in the adult primate. Partially supported by USPHS Grant 1 F32 NS 06179-01.


As with many other genitourinary organs, the structure of the clitoris has not been studied in any detail. The clitoris is formed by two connective tissue sheaths, the urethral being free from any overlying epithelium, the vestibular lying on the ischiocavernous. Although there is no corpus spongiosum the clitoris is considered to be the "homologue" of the penis in the male. The existing data is insufficient to ascertain how this functional distinction is manifested since histological data is not available in the current literature.

The data for the study was obtained from female cynomolgus monkeys and from patients undergoing a partial clitoridectomy for hormone-related hypertrophy of the clitoris. Each sample was divided into two parts - (1) the clitoris being free from any overlying epithelium for use in various light microscopic analyses, and (2) the portion being fixed immediately by immersion in glutaraldehyde for EM study. Light microscopic techniques utilized included hematoxylin and eosin, phosphoarginic acid (AChE) and glyoxylic acid (GA) histofluorescence. In addition to routine EM preparation, Wood's (1963) glutaraldehyde-chromatochrome method was employed for specific localization of adrenergic vesicles.

Preliminary tests indicate that the primates clitoris is composed of loose connective tissue with occasional vein-like spaces. Collagen bundles and elastic fibers, along with the connective tissue sheaths make up the bulk of the clitoris. Smooth muscle cells are rarely present, except in the walls of blood vessels. Nerve bundles of various sizes, containing both myelinated and unmyelinated nerve fibers, and small blood vessels can be found traversing the clitoris parallel to the longitudinal axis of the organ. Pacinian and Meissner's corpuscles appear frequently in the subcutaneous layers.

Presumed adrenergic (GA-fluorescent) fibers are found most often forming plexus around the small blood vessels, although a few appear in the walls of the minor blood vessels. Possible cholinergic fibers (AChE-positive) are found more frequently in the stroma and in small and large nerve fiber bundles. EM analysis of the neural elements supporting these light microscopic results.

The above data suggest that the morphology of the clitoris is significantly different from that of the penis and thus the two are not truly homologous in the adult primate. Partially supported by USPHS Grant 1 F32 NS 06179-01.
The frequency of discharge of neurons in the preoptic region of many lower vertebrates and mammals is highly sensitive to changes in peripheral and central temperature. These neurons are implicated in thermoregulation. Microstructural neural patterns may be involved in temperature sensing and/or integration necessary for temperature regulation in mammals. Extracellular recordings were made from single cells in medial and lateral preoptic regions of green sunfish, *Lepomis cyanellus*, while altering forebrain and skin temperatures. Warm sensitive (274) and cold sensitive (64) cells were identified; some of these responded to forebrain temperature changes, some to skin temperature changes and others to both forebrain and skin temperature alteration. In addition, some temperature-insensitive (724) cells were found. However, examinations of interspike interval patterns of these grossly insensitive cells showed complex alterations from unimodal firing patterns to bimodal patterns. These transitions, which consisted of changes from a continuous random firing pattern to a bursting pattern, occurred at different specific temperatures for different units; in one 25°C acclimated fish, neurons were found which showed transition at 17°C. The temperatures of transition of different cells appear to comprise a continuum in all fish examined. Such a system of neurons may provide a coded information for behavioral thermoregulation. It is suggested that microstructural coding of temperature may be a property found in lower vertebrates as well as in mammalian investigation of the general temperature alterations on firing patterns of preoptic neurons is in progress.

Supported by NSF PCM 75-15861 and NIMH PHS GM07143.

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**SOME CONNECTIONS OF THE SKATE DORSAL AND MEDIAL PALLIA.**


Some pallial connections in adult thornback skates (*Platyrhinoides triiseriata*) were revealed with HRP histochemistry (DIAB or TRITC or TMB substrates). Animals survived 8-14 days at 16°C after unilateral pallial injections of 200-400 nl of Sigma VI HRP (20-40%). Retrograde and anterograde transport were apparent. HRP-positive neurons were present in both an ipsilateral and contralateral rostral tbalamic nucleus following pallial injections confined to the telencephalic central nucleus and dorsal pallial lamination lying immediately dorsal to the central nucleus. Anterograde fibers could be followed over the caudal pole of the hemisphere where they turned caudally to terminate in the ipsilateral rostral thalamic nucleus that projects to the central telencephalic roof. Fibers were also followed from the roof ventrally and caudally where they decussate dorsal to the optic chamber and terminate in the contralateral rostral thalamic nucleus. The rostral thalamic nucleus projecting to the central telencephalic roof has been identified as the lateral geniculate in sharks such as *Squalus acanthias* (Schroder & Edsborn, 1974). In sharks the LGN projection appears totally crossed, as are the primary optic projections. Skates, however, possess ipsilateral as well as contralateral retinotrophic projections (Northcutt & Board, unpublished observations) and this difference may account for the bilateral projection of the rostral retinotrophic projection in skate pallial injections with greater rostro-caudal extent reveal HRP-positive neurons in an ipsilateral cell group immediately dorsal to area superficialis balls, and in the telencephalic central and caudal inferior lobes. Larger injections also revealed two additional cell groups more caudal in the thalamus. The more dorsal group probably corresponds to the central thalamic nucleus of Edsborn; the second nucleus is more ventromedial and has not been previously named. These larger pallial injections appear to include parts of the medial pallium and reveal pallial efferents comparable to those reported by Edsborn in sharks (CBP, 1972). Telencephalic efferents terminate bilaterally on the septal nuclei as well as on the caudal hemisphere where they terminally. Some of these efferents descend into the habenular commissure and supraoptic decussation. Fibers appear to terminate bilaterally in the habenular nuclei, dorsal thalamus, optic tectum, and tegumentum. Descending fibers were also traced to the contralateral lateral line and functional terminals in lateral insensate terminal coordinations occur.

(Research was performed in accordance with NIH Guidelines Vol. 7, 1978. Supported part by MIN Grant 15109 and Rackham Faculty Research Grant (UM) to RGN.)

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**IDENTIFICATION OF HOMOLOGOUS MUSCLES AND MOTONEURONS IN 2 SPECIES OF SANDCRAB BELONGING TO DIFFERENT TAXONOMIC FAMILIES SUGGESTS ANCESTRY OF SWIMMING BY UROPOD BEATING.**


Some pallial connections in adult thornback skates (*Platyrhinoides triiseriata*) were revealed with HRP histochemistry (DIAB or TRITC or TMB substrates). Animals survived 8-14 days at 16°C after unilateral pallial injections of 200-400 nl of Sigma VI HRP (20-40%). Retrograde and anterograde transport were apparent. HRP-positive neurons were present in both an ipsilateral and contralateral rostral tbalamic nucleus following pallial injections confined to the telencephalic central nucleus and dorsal pallial lamination lying immediately dorsal to the central nucleus. Anterograde fibers could be followed over the caudal pole of the hemisphere where they turned caudally to terminate in the ipsilateral rostral thalamic nucleus that projects to the central telencephalic roof. Fibers were also followed from the roof ventrally and caudally where they decussate dorsal to the optic chamber and terminate in the contralateral rostral thalamic nucleus. The rostral thalamic nucleus projecting to the central telencephalic roof has been identified as the lateral geniculate in sharks such as *Squalus acanthias* (Schroder & Edsborn, 1974). In sharks the LGN projection appears totally crossed, as are the primary optic projections. Skates, however, possess ipsilateral as well as contralateral retinotrophic projections (Northcutt & Board, unpublished observations) and this difference may account for the bilateral projection of the rostral retinotrophic projection in skate pallial injections with greater rostro-caudal extent reveal HRP-positive neurons in an ipsilateral cell group immediately dorsal to area superficialis balls, and in the telencephalic central and caudal inferior lobes. Larger injections also revealed two additional cell groups more caudal in the thalamus. The more dorsal group probably corresponds to the central thalamic nucleus of Edsborn; the second nucleus is more ventromedial and has not been previously named. These larger pallial injections appear to include parts of the medial pallium and reveal pallial efferents comparable to those reported by Edsborn in sharks (CBP, 1972). Telencephalic efferents terminate bilaterally on the septal nuclei as well as on the caudal hemisphere where they terminally. Some of these efferents descend into the habenular commissure and supraoptic decussation. Fibers appear to terminate bilaterally in the habenular nuclei, dorsal thalamus, optic tectum, and tegumentum. Descending fibers were also traced to the contralateral lateral line and functional terminals in lateral insensate terminal coordinations occur.

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**COMPARATIVE ORGANIZATION OF REPTILIAN RETICULAR FORMATION.**


The brains of species of the genera *Crotalus*, *Agkistrodon*, and *Heterodon*, lizards of the genera *Lampropeltis*, *Resplendens*, and *Gekko*, turtles of the genera *Chrysemys*, *Pseudemys*, and *Terrapene*, and the crocdilidian *Caiman* were processed using Golgi and histological techniques.

The reticular formation in reptiles is divisible into several distinct fields: an inferior reticular field (RI) in the myelencephalon, a middle reticular field (RM) in the caudal two-thirds of the metencephalon, and a superior reticular field (RS) in the rostral third of the metencephalon. A dorsolateral-ventrolateral metencephalic reticular field (MDL-MVL complex) is present in all but isomeric levels. The inferior raphe nucleus (RaI) is identified with the reticular formation, as is the middle raphe nucleus (RaM).

In lizards, snakes and crocdilolians, RaI is subdivided into dorsal vs. ventral portions (RID vs. IVI). Turtles possess only the RID field. In all the reptiles studied, RM, RS, the Nueral complex could be identified in T. boeleni. The raphe nuclei varied considerably. In snakes, only mediumsized neurons are present in RaI, whereas RaI in lizards and turtles contains large cells. RaI in crocdilolians contains giant cells.

Small reticular neurons (~30µ) have fusiform or triangular soma which have three to six sparingly-branching dendrites which average 236µ in length. Dendrites of large neurons in RID show a particular orientation. Dendrites of RaM course horizontally. Most dendrites of large neurons in RM course ventrally. RM neurons bear dendritic aberrations which show the dorsoventral width of the brain stem, and the size of the medial longitudinal fasciculus dorsally and touching the pia mater ventrally. Some large RM neurons bear dendrites which cross the midline and terminate in the contralateral RM.

The dendrites of both small and large reticular neurons are devoid of exsiccations and ramify predominantly in the transverse planes.

The rettilliam reticular formation resembles that of mammals in that it consists of distinct sub-nuclei or fields which contain specifically branching, functionally distinct, efferent systems. An unexpected finding was the wide variation observed in the organization of the raphe complex in the various reptilian groups.
QNB was localized in high concentrations in the neuropil of several auditory nuclei, nucleus ovoidalis, dorsal pallium (paleostriatal complex, PC), the presumed equivalent of the mammalian basal ganglia. Using catecholamine (CA) histochemistry, acetylcholinesterase (AChE) histochemistry, and tritiated quinuclidinyl benzilate (QNB), a specific muscarinic cholinergic receptor, the presumed homologue of the mammalian basal ganglia were recognized in the turtle (Chrysemys scripta). 1) A small-celled zone (paleostriatal augmentum, PA) occupies most of the basolateral telencephalic wall, extending from the olfactory tubercle to the amygdaloid region. The neuropil of PA is rich in AChE, weakly positive for substance P and contains a fine network of terminal axons terminating on to that of the olfactory bulb of mammals. 2) A medial small-celled area (area d, Riss, Halpern and Scalia, BB and E, 1969), coextensive with PA along the rostrocaudal axis, is contiguous with the olfactory tubercle. The neuropil of area d is also rich in AChE, but differs from that of PA in that it is more intensely positive for substance P and contains a network of thick CA fibers and terminals. 3) A group of large AChE-rich neurons (globus pallidus, GP) is interspersed with the fibers of the lateral forebrain bundle throughout the intermediate PC levels. The neuropil of GP is rich in substance P, but nearly devoid of CA and AChE.

The efferent projections of the PA were examined using autoradiographic tracing techniques. The most prominent projection of the PA was to a tegmental cell field containing numerous CA neurons. Other targets of the PA included several subthalamic nuclei, the dorsal nucleus of the posterior commissure, a few CA nuclei of the ventromedial thalamic nucleus. Injection of horseradish peroxidase into the tegmental CA cell field indicated that the projection to this region from PA arises from GP and caudal areas D. Only a few neurons were labeled by such injections. Other regions injected (Parent, B. Res., 1976) have shown that PA receives an extensive input from the tegmental CA cell field. The projection of the PA upon the tegmentum is reminiscent of the projections of globus pallidus and nucleus accumbens upon the tegmentum in mammals. The present connectional and histochemical data are consistent with the hypothesis that PA, GP and area d in turtles are similar to caudoputamen, globus pallidus and nucleus accumbens, respectively, in mammals. I thank Harvey Karten for his support during this research. Supported by USPHS 1 F 3 NS 05682001.

EVIDENCE FOR CHOLINERGIC MECHANISMS IN BRAIN REGIONS RELATED TO BIRD SONG. Susan M. Ryan and Arthur P. Arnold. Dept. Psychol. Neurosciences Program and Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Brain regions thought to be involved in the control of song in the zebra finch, Poephila guttata, were examined histochemically using the Karnovsky and Roots direct-coloring method for the detection of acetylcholinesterase (AChE). In order to control for the possible presence of nonspecific cholinesterases, two substrates, acetylthiocholine and butyrylthiocholine, and several enzyme inhibitors, tetraiodopropyl pyrophosphoramide (iso-OPA), benzilic acid, and acetone, were included in the incubations. Manipulations with these compounds demonstrated that most of the reaction product with the substrate acetylthiocholine was AChE. In addition, some of the subjects were pretreated with the cholinesterase inhibitor, diisopropylfluorophosphate (DFP). DFP irreversibly inactivates AChE, which allowed observation of newly synthesized AChE in cell bodies and processes. Several brain areas thought to be involved in vocal control in adult males and females contain AChE. These nuclei include Area X (or dorsolateral LPO), a large, neocortical nucleus of the anterior neornitretium (MAN), nucleus Interface (NI), central nucleus of the ventral hyperstriatum (VHC), intercollicular nucleus (ICo), robust nucleus of the archistriatum (RA), and tracheosyringeal nucleus (of the hypoglossal nerve nucleus [XII]). In addition, several auditory nuclei, nucleus ovoidalis, dorsal portion of the lateral mesencephalic nucleus (MLd), and field L also contain AChE. Of the visual control nuclei, Area X, NI, ICo, ICc, and X II contain intense reaction product, though the surrounding tissue. All of the vocal control nuclei, except Area X, contain small, intensely labeled cell bodies; however, in Area X an extremely dense reaction product is present in the neuropil, suggesting possible cholinergic input. Autoradiographic procedures localize binding of tritiated quinuclidinyl benzilate (QNB), a specific muscarinic cholinergic antagonist, in the brain. In both males and females, QNB is localized to high concentrations in the neuropil and cell bodies of the medial pallium, Area X (or dorsolateral LPO). This binding was displaced by pre-injection with two specific muscarinic antagonists, atropine sulfoxide and scopolamine hydrobromide, and by saline, indicating that the binding is of limited capacity and specific to muscarinic cholinergic receptors. Taken together, the high concentrations of AChE and high binding of QNB in Area X strongly implicate cholinergic mechanisms in the function of Area X. Supported by NSF grant BNS 77-05973 to A.P.A.
THE EFFECTS OF UNILATERAL LESIONS AND IPSILATERAL OR CONTRALATERAL EYE CLOSURE ON THE SOCIAL BEHAVIOR AND ACTIVITY LEVELS OF THE WESTERN FERNS LIZARD. Robert S. Ten
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Street, Chicago, Illinois 60615.

Dorsal striatal lesions were studied in Sceloporus occidentalis before and after unilateral telencephalic lesions noting the effects after closing either the ipsilateral or contralateral eye. An adult male animal was placed in a large, enclosed, natural environment. The animal occupied the territory alone for 48 hours, then another adult male was introduced. Activity levels (number of times changing position and number of times changing location) and the ophthalmic behavior (amount of assertion displays and challenge displays) were recorded. The animals were removed and the resident was either sham lesioned, lesioned in the anterior DVR, lesioned in the paleostriatum (nucleus accumbens) or lesioned in the amygdala. All lesions were unilateral. The resident’s ipsilateral (to the lesion) or contralateral eye was shutted shut, he was reintroduced into the territory with the observation period repeated. The animals were again removed and the eye was opened and the other eye shutted shut. Again, 48 hours later the observations were repeated. Animals showing normal activity were observed for at least one additional observation period. The intruding lizard was the same animal for all the residents. Light, temperature and diurnal activity cycles were all held constant.

Dramatic lesioning and anterior DVR lesions did not result in a change in activity level or agonistic behavior through either eye. Certain small lesions in the paleostriatum and/or amygdala, resulted in a drop in activity and challenge behavior when the contralateral eye was open. Challenge displays were entirely absent, assertion displays nearly absent, activity was reduced 30% or more and dominance in the territory was minimal. The animal could still move, however, as assessed by their normal visual predatory behavior. When the ipsilateral eye was being used social behavior and activity was either normal or reduced but since dominance displays were always present, the animal was always dominated by the other eye.

The observations confirm the role of the reptile telencephalon in arousal, attention and agonistic behavior and the functional effect of nearly complete crossing of optic fibers. They further suggest that in these animals the mechanism of arousal is heavily dependent on visually processed information related to social behavior.

471. DISCRIMINATION OF MIRROR-IMAGE SYMMETRICAL STIMULI AFTER LESIONS OF THE VISUAL SYSTEM IN PIGEONS. Susan R. B. Weiss and William Modos. Dept. Psych., Univ. of Maryland, College Park, MD 20742

Mammals have been reported to have greater difficulty discriminating between stimuli that differ by one dimension (e.g., lateral vs. symmetrical, vertical vs. symmetrical, or right vs. left) than stimuli with similar but opposite form. We have trained pigeons to differentiate between stimuli that differed by one dimension (e.g., lateral vs. symmetrical) and one that differed by another (e.g., vertical vs. horizontal).

Following training, bilateral stereotactical lesions were made either in visual wulst, which is a telencephalic component of the thalamofugal visual pathway or eoctostriatum, which is a telencephalic component of the tectofugal visual pathway. After surgery, both groups were retrained to the preparative criterion. Visual stimulus lesions resulted in minor, transient deficits on all three stimulus pairs. Mi-symmetrical stimuli showed no greater impairment than non-Mi stimuli. Lesions of eoctostriatum resulted in moderate to severe impairment in the discrimination of all three stimulus pairs. All subjects with eoctostriatum lesions exhibited a greater impairment in the discrimination of lateral Mi-symmetrical stimulus than the vertical Mi-symmetrical or unsymmetrical stimuli.

Intact mammals require more training to discriminate pairs of Mi stimuli than any other type of stimulus pair differing only in spatial orientation. In contrast, intact pigeons learn to discriminate Mi and non-Mi stimuli at the same rate. Interruption of the tectofugal visual pathway in pigeons results in a difference significantly greater impairments in the lateral Mi stimulus than non-Mi stimuli. The differences between birds and mammals in their ability to discriminate lateral Mi stimuli may be related to differences between them in the organization of their hemispheric interconnections.


In bullfrogs (Rana catesbiana) efferents from the thalamic nuclei receiving tectal and tectal projections terminate heavily in the striatum (Wilczynski and Northcutt, Anat. Rec., 193: 721, 1979). In order to determine routes by which the striatum in turn influence midbrain sensory structures, we investigated striatal efferents in adult bullfrogs using autotographic and degeneration techniques, and the anterograde transport of horse-radish peroxidase (HRP). Results from each technique were essentially identical. Except for the small ventral enucleation of the lateral pallium, no, all areas nuclei and major projection fields of the striatal efferents descend in the lateral forebrain bundle (LFB) through the anterior entopeduncular nucleus (AE) where a large fascicle leaves the LFB to cross in the anterior commissure and terminate in the homologous cell groups in the medial geniculate body of the thalamus.

These observations confirm the role of the reptile telencephalon in arousal, attention and agonistic behavior and the functional effect of nearly complete crossing of optic fibers. They further suggest that in these animals the mechanism of arousal is heavily dependent on visually processed information related to social behavior.


Concepts of homology in the central nervous system of different species are critical to comparative neurology. All studies of neuroscience rely on such concepts in relating the significance of their findings. Yet there are few critical evaluations of its definition and practical application. The present study explores the premise that the shapes of neurons and axons can be used to demonstrate homologous cell groups in the medial geniculate body of the opossum and cat. Morest (Anat. Rec., 1965, 151, 390) compared the structure and connections of the neurons with the Golgi and Nauta methods to derive homologous nuclei. We have confirmed and extended this work. In the ventral division, the thalamo-cortical neuron in both species has tufted dendrites which are arranged in parallel laminae. A smaller Golgi type II cell with a locally arborizing axon is also present. In the dorsal division, the opossum has two types of principal neurons, as does the cat, and there is close morphological correspondence between the species; in the cat, there are also two kinds of Golgi type II cells, while the opossum probably has only one type. In the medial division, both species have numerous (as many as five) cell types, including Golgi type II cells, and a corresponding richness of afferent inputs. A major difference between the species is the relative abundance of Golgi type II cells in the cat and their pacity in the (and) each of the thalamic subdivisions. In addition, certain subdivisions are proportionately expanded or elaborated in the cat (e.g., pars lateralis of the ventral division), while others are relatively more prominent in the opossum (e.g., pars ovoidalis of the ventral division.) We conclude that to these concepts, the topographical position of nuclei, their developmental history or functional organization are insufficient to confirm homology in the nervous system; while they can be useful in the determination of homology, we propose that a more reliable criterion is neuronal structure.

Supported by USPHS grants 1 F32 NS05485 and 5 R01 NS14347.
DEVELOPMENT
CONSTANCY OF SPATIAL PREFERENCES OF GRASSHOPER MOVEMENT DETECTOR NEURONS DURING POST-EMBRYONIC EYE DEVELOPMENT.

Anthony Altar*, Mark F. Nelson*, Carla S. Whitacre*,
Edwin Meyer, Jr.*, and Loy D. Lytle.  Department of
Psychology, University of California, Santa Barbara, CA 93106.

The biosynthesis of a variety of monoaminergic neuro-
transmitter compounds in fetal brain depends on the relative availability of precursor com-
pounds to brain or peripheral neurons. For example, dietary or pharmacological manipulations which increase the brain concentrations of L-tyrosine produce parallel increases in the rates of synthesis of the catecholamine neurotransmitters dopamine and norepinephrine [C. J. Gibson and R. J. Wurtman, Biochem. Pharmacol. 26: 1117 (1977)]. Similarly, injections of L-tyrosine into previously untreated fetal rats increase the sizes of certain brain regions (H. Ishida and M. Saito, J. Neurosci. (1977)) and also produce dose- and time-related increases in the brain concentrations of the indoleaminergic neurotrans-
mitter serotonin (T. M. Cowan, J. Neurosci. 1: 149 (1977)). In the present set of experiments we were interested in determining the possible extent to which alterations in the availability of precursor amino acids might cause changes in the abilities of fetal animals to synthesize brain neurotrans-
mitting enzymes.

To accomplish this goal, eight day post-conception pregnant albino rats were fasted overnight, and then injected with 80 mg/kg of L-tyrosine (10 ml/kg of 0.9% saline, pH 10.4). Animals were killed 30, 60, or 120 min post-injection and tyrosine concentrations were determined in the cerebral cortex, pons, and midbrain of the fetuses using a fluorometric assay. Dopamine and norepinephrine concentrations in fetal brain tissues were also measured using the radiometric-enzymatic assay of J. T. Coyle and D. Henry [J. Neurochem. 21: 61 (1973)]. Tyrosine concentrations increased in a dose-related fashion: All tissues examined for up to 60 min post-injection, and return to control values by 120 min. Fetal brain concentrations of dopamine and norepinephrine increased by 30 min or 60 min, respectively, following the maternal tyrosine injections. These results indi-
cate that the peaks of syntheses of these neurotransmitters are not co-ordinated with the other tyrosine concentrations, and the results suggest that the brain tissues may be regulated, at least in part, by the relative availability of precursor compounds. (Supported in part by a grant from NIH).

RESPONSE OF INFANT RAT BRAIN TO CHANGES IN PLASMA CONCENTRATIONS OF AMINES DURING DEVELOPMENT.

Gary Mihailoff and Donald J. Woodward (SPON: D. J. Woodward).


DEVELOPMENT

DETECTOR NEURONS DURING POST-EMBRYONIC EYE DEVELOPMENT.

The head of the locust, Schistocerca gregaria, bears a population of wind-sensitive hairs whose inputs are used for the initiation, maintenance, and control of flight. The hairs themselves are physiologically and morphologically indistinguishable, but hairs in different locations on the head form different neuronal projections within the c.n.s.: hairs on the side of the head send axons to the c.n.s. via the trigeminal nerve, and to a small extent the dorsal tegumentary nerve, and form entirely ipsilateral projections within the suboesophageal ganglion and thoracic ganglia; hairs on the top of the head send axons to the c.n.s. exclusively through the dorsal tegumentary nerve and form additional contralateral branches and arborizations in the suboesophageal ganglion and thoracic ganglia.

Are these patterns of projection determined by factors intrinsic to the sensory receptors themselves, or do they primarily depend upon later interactions between their axons and the environment through which they grow in the c.n.s.? The hairs developed from the grafts by cobalt-filling individual neurons and viewing them in wholemount preparations.

Hairs developing from the grafts were encircled by host hairs developing from the surrounding host epidermis. The graft hairs now grew from the epidermis in association with the surrounding host axons and entered the c.n.s. at the host site via the host nerve. However, once the graft axons were in the c.n.s., in no case did they behave according to their altered environment. Rather they all formed projections which were entirely appropriate to their epidermis of origin.

We conclude that receptor cells are assigned, on the basis of early decisions taken by their epidermal cell precursors, an intrinsic developmental programme which specifies their projection patterns.

Enduring morphological alteration of hippocampus and cerebellum in rats prenatally-exposed to ethanol. David F. Barnes, Don W. Walker and Steven F. Zornettier. Dept. of Neuroscience, Univ. of Florida College of Medicine and VA Medical Center, Gainesville, FL 32610.

Pregnant long-evans rats were maintained on an ethanol containing diet (40% ethanol-derived calories; 9.7% v/v ethanol) during a 22% of gestation. The grafts were paired-fed a sucrose-containing liquid diet with sucrose substituted isocalorically for ethanol or given free access to pelleted lab chow and water. In the first experiment, prenatally ethanolized pups were culled and the brains were removed and coded to determine the number of hippocampal pyramidal cells was determined in cerebellar Purkinje cells. In addition, there was a decrease in hippocampal pyramidal cells (9%) which may be a result of the loss of Purkinje cells rather than the exposure to ethanol per se. The results indicate that prenatal ethanol exposure results in an apparently permanent reduction in the number of hippocampal and cerebellar neurons.

Enduring morphological alteration of hippocampus and cerebellum in rats prenatally-exposed to ethanol. David F. Barnes, Don W. Walker and Steven F. Zornettier. Dept. of Neuroscience, Univ. of Florida College of Medicine and VA Medical Center, Gainesville, FL 32610.

The number of hippocampal pyramidal cells was determined in hippocampal and cerebellar neurons from newborn rat nodose ganglia when grown in culture. All neurons were grown in the presence of NMF, by 24 hrs they attached to the substrates and extended processes. We plated 30 neurons under phase contrast microscopy and in the virtual absence of non-neuronal cells (2) on a preformed layer of skeletal myotubes or myocytes. We recorded from these neurones for up to 10 days post-plating. Neuronal activity (1) many neurons had spontaneous excitatory postsynaptic potentials (epsps). In addition, in about 20% of randomly chosen neuron pairs in cultures stimulated with one neuron, the evoked epsp's were reversibly blocked by standard cholinergic antagonists indicating that the synapses are cholinergic. In contrast spontaneous or evoked epsp's were much rarer events in sister cultures grown in condition (2); they were detected in less than 5% of pairs tested. Some factor accounts for the failure of spontaneous or receptor-neuron-pairs to form cholinergic synapses in the absence of cholinergic receptors. We tested neuronal sensitivity to acetylcholine (ACh) primarily by pressure ejection (similar results were obtained by either bath or iontophoretic application). For each culture dish, which usually contained approximately 500 neurons, we routinely tested 50 neurons chosen at random and scored each one for either sensitivity or insensitivity to ACh. The majority of ACh used a sensitive neuron was one which depolarized at least 5 Venice, though ACh had no effect or a small effect on about 40 neurons. We then performed experiments where sister cultures were grown either alone or on muscle, and found that neurons grown on muscle showed enhanced sensitivity to ACh in the absence of other cells. The majority of neurons grown on muscle and fed every 2 days were sensitive to ACh whereas neurons on the same substrates grown every 3 days were not sensitive. Feeding the 'neurons - alone' cultures every 4 days does not give as many sensitive neurons (50-70%, n = 500) compared to those fed every 2 days, however, the proportions are still significantly different from sister cultures grown on muscle.

We are investigating what factors may be involved in the expression of ACh receptors as well as the pharmacology of these receptors and whether the neurons are sensitive to other transmitters and substances. (Supported by the Whitney Foundation, the American Dysphoria Association and the American Heart Association 78564).


Cholinergic synapses form between dissociated neurons from newborn rat nodose ganglia when grown in culture. The formation of these functional synapses (as well as sensitivity to the neurotransmitter acetylcholine (ACh) receptors depends on the environmental conditions in which these cells are grown. Nodose ganglia (an autonomic sensory ganglion) were dissociated from the newborn rats and mechanically dissociated. All neurons were grown in the presence of NMF, by 24 hrs they attached to the substrates and extended processes. We plated 30 neurons under phase contrast microscopy and in the virtual absence of non-neuronal cells (2) on a preformed layer of skeletal myotubes or myocytes. We recorded from these neurones for up to 10 days post-plating. Neuronal activity (1) many neurons had spontaneous excitatory postsynaptic potentials (epsps). In addition, in about 20% of randomly chosen neuron pairs in cultures stimulated with one neuron, the evoked epsp's were reversibly blocked by standard cholinergic antagonists indicating that the synapses are cholinergic. In contrast spontaneous or evoked epsp's were much rarer events in sister cultures grown in condition (2); they were detected in less than 5% of pairs tested. Some factor accounts for the failure of spontaneous or receptor-neuron-pairs to form cholinergic synapses in the absence of cholinergic receptors. We tested neuronal sensitivity to acetylcholine (ACh) primarily by pressure ejection (similar results were obtained by either bath or iontophoretic application). For each culture dish, which usually contained approximately 500 neurons, we routinely tested 50 neurons chosen at random and scored each one for either sensitivity or insensitivity to ACh. The majority of ACh used a sensitive neuron was one which depolarized at least 5 Venice, though ACh had no effect or a small effect on about 40 neurons. We then performed experiments where sister cultures were grown either alone or on muscle, and found that neurons grown on muscle showed enhanced sensitivity to ACh whereas neurons on the same substrates grown every 3 days were not sensitive. Feeding the 'neurons - alone' cultures every 4 days does not give as many sensitive neurons (50-70%, n = 500) compared to those fed every 2 days, however, the proportions are still significantly different from sister cultures grown on muscle.

We are investigating what factors may be involved in the expression of ACh receptors as well as the pharmacology of these receptors and whether the neurons are sensitive to other transmitters and substances. (Supported by the Whitney Foundation, the American Dysphoria Association and the American Heart Association 78564).

A posteriori to anterior gradient in variability of the structures of two identified neurons in crayfish. Michael Bastiani and Brian Muloney. Depts. of Physiology and Zoology, University of California at Davis, Davis, CA 95616.

The flysh muscle receptor organs (MRO's) monitor the position and movement of the abdomen. A pair of receptors, tonic (MRO1) and phasic (MRO2) is associated with each abdominal hemiganglion. The sensory neurones from these ganglia are identified as the SN's from the suboesophageal ganglion and thoracic ganglia; hairs on the top of the head are sensitive to wind and have a characteristic structure. These receptors are sensitive to other transmitters and substances. (Supported by the Whitney Foundation, the American Dysphoria Association and the American Heart Association 78564).
DEVELOPMENT


When a food deprived neonatal rat receives a small infusion of milk in its mouth via an intragastic cannula, it ingests a substantial amount of the diet and also shows a dramatic behavioral activation. The feeding and activation only occur when the pup is maintained in a warm environment (Hall, 1978, in press). The present study determined whether body temperature or perceived ambient temperature was the basis for the temperature dependence of early ingestive responding.

Fine polyethylene intragastic cannulas, through which diet could be infused, were implanted in the mouths of six-day-old Charles River CD strain rats. Prior to testing, the body temperature of one pair of pups was adjusted to 33-34°C (for Warm Temperature pairs), while the temperature of another pair was adjusted to 28-29°C (for Cool Temperature pairs). One of the pups in each pair was tested at room temperature (26°C) and the other was tested in a warm (34°C) incubator. A pulse of milk was infused into the pup's mouth through the cannula once a minute for 3 min. Every 30 sec, the pup's activity was recorded and a variety of behaviors, including probbing and mouthing, were scored. Intake was determined by measuring the change in body weight from the beginning to the end of the test. Warm pups (n = 7 pairs) tested in a warm environment had significantly higher levels of activity, mouthing, probbing and intake than cool pups tested in a cool environment. Similarly, cool pups (n = 7 pairs) tested in a warm environment were more active, showed more mouthing and probbing, and ingested more diet than cool pups tested in a cool environment (see Table). Together, these results indicate that the effect of infused diet on the behavioral activation and intake of a deprived pup depends on both contextual cues (e.g. ambient temperature) and physiological conditions (e.g. body temperature).

<table>
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<tbody>
<tr>
<td>Intake (% of Infused vol.)</td>
<td>93 ± 7</td>
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<td>Activity</td>
<td>11.1 ± 6</td>
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(Supported by USF BNS 77-23508 & N.C. Div. of Nental Health.)


A new neuronal growth factor(s), derived from medium conditioned by mouse neuroblastoma cultures, enhances survival and stimulates development of mammalian autonomic neurons. The factor causes a 2-3 fold increase in neurite outgrowth and tyrosine hydroxylase activity of embryonic mouse superior cervical ganglion (SCG) cultured without added nerve growth factor (NGF). Dose-response analysis revealed that these responses occurred as a saturable function of factor concentration. In addition, choliner acetyltransferase activity was also increased in the cultured SCG by the factor. Antiserum to NGF did not inhibit growth responses to the new factor, suggesting that it was separate and distinct from NGF. Moreover, the new factor enhanced survival of parasympathetic and sympathetic neurons in cell culture, but not sensory neurons clearly distinguishing the factor from NGF. These results suggest that heart cells in culture are capable of elaborating a new growth factor which other cells respond to by survival and differentiation of sympathetic and parasympathetic neurons.

(This work was supported by grants from the NIH, National Science Foundation, by Autonomia Foundation Inc. and Irma T. Hirsch Trust Fund.)

484 DIFFERENTIAL EFFECTS OF D-Amphetamine ON LOCOMOTOR ACTIVITY IN IMMATURE AND MATURE RATS. Richard H. Bauer, Department of Psychology, Kansas State University, Manhattan, KS. 66506.

In altricial species, such as the rat, biochemical and histological studies indicate that catecholamine containing neuronal cell bodies in the lower brain stem are nearly fully developed at birth. During development, axons from these norepinephrine (NE) and dopamine (DA) containing cell bodies grow in a rostral direction, such that successively higher structures are innervated. In the rat, innervation of cortical areas occurs at about 45 days of age.

Since ascending catecholamine neurons in the rat are developing from birth to about 45 days of age, drugs which alter behavior by acting on catecholamines would be expected to have differential behavioral effects at different maturational stages. D-amphetamine is thought to alter locomotor activity and induce stereotyped behaviors by presynaptically increasing the release and reducing the reuptake of NE and DA. Thus, D-amphetamine would be expected to have differential effects on locomotor activity of immature and mature rats. This hypothesis was tested in the present study by recording photo-cell crossings of 15-, 17-, 21-, 36-, 93-, and 275-day-old rats injected with either physiological saline or 0.5, 1.0, 4.0, 8.0, or 16.0 mg/kg of D-amphetamine sulfate. Photo-cell crossings were recorded starting immediately after the injection for a period of 4 hours. The two oldest groups were tested in a 45 x 45 x 45 cm Plexiglas chamber. All apparatus dimensions for younger groups were reduced according to the mean snout to rump length.

For each animal, the number of photo-cell crossing during 15-min intervals was determined. Statistical analyses showed that only the 0.5 mg/kg dose resulted in a slight increase in photo-cell crossings in the two youngest groups. In 21 and 36-day-old rats, there was a positive relationship between drug dose and photo-cell crossings. In the two oldest groups, lower doses increased photo-cell crossings, whereas higher doses reduced crossings. The temporal changes occurring during the 4-hour session also differed as a function of development.

In a second experiment, para-hydroxyamphetamine, which has only peripheral nervous system effects, did not alter photo-cell crossings of immature or mature rats.

The present findings are consistent with the hypothesis that, because of differences in maturation of catecholamine neurons, catecholaminergic drugs would produce differential behavioral effects in immature and mature rats.

486 CELL DEATH IN THE DEVELOPING HAMSTER SUPERIOR COLLICULUS. Anne T. Berg* and Barbara L. Finlay Department of Psychology, Kansas State University, Manhattan, KS. 66506.

Degenerating cells may be observed with light microscopy in the superior colliculus of the golden hamster during the first nine postnatal days. The number of degenerating cells observable at any point is small. In the period of maximum rate of cell death, postnatal days 3-9, 4-5 degenerating cells can be found per 1000 normal cells; by day 8, only 1-2 degenerating cells per 1000 are visible. As in chick (1) and rat (2), this period of cell death follows neuronal differentiation in the tectum and is coincident with the major period of axon ingrowth and outgrowth.

Within the tectum, the distribution of degenerating cells is fairly uniform. The rate and time course of cell death in tectum may be distinguished from the rate and time course in surrounding structures and other visual structures. 1. Cantino and Danee, '72 2. Areas and Astrum, '77

Supported by NSF Grant BNS 77-07066

Morphological analysis of a developing visual system has been undertaken at the EM level in an attempt to elucidate cellular interactions operative in the formation of the retina. Development of the zebrafish (Brachydanio rerio) project approximately 50,000 fibers from each eye to the contralateral optic tectum. Staged embryos, with relatively few (about 1800) fibers leaving the eye provide the opportunity to examine cells forming the connection to the tectum. Developing cells in the visual system have been reconstructed from serial section electron micrographs using the CARTOS computer graphics system. In the retina, small clusters of adjacent ganglion cell somata spin out axons which, over long distance in the optic nerve, maintain their contiguity. Growth cones of these cells confine their activity to neighboring axonal surfaces.

The retinal surface maps in a systematic fashion into the cross sectional profile of the optic nerve. Cell position in the retina can be defined by polar coordinates (r,θ), with the choroidal fissure at θ=0 and r=0 at the optic nerve head. In the optic nerve, radial position transforms into the dorso-ventral axis, while angular position transforms into the nasal-temporal axis.

The present study suggests that the optic nerve develops as a highly ordered structure capable of carrying the projection back to the tectum, such organization is mediated by interactions between and among growing ganglion cell axons which originate from the retina. Growth cones of these cells can be defined by polar coordinates (r,θ), with the choroidal fissure at θ=0 and r=0 at the optic nerve head. In the optic nerve, radial position transforms into the dorso-ventral axis, while angular position transforms into the nasal-temporal axis.

Proliferation of granule cell precursors in rat hippocampus is inhibited by hydrocortisone (HC). A neuroanatomical study. Martha C. Rohr* (SPON: Kent Kent), Dept. Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT. 06226.

Postnatal genesis of microglioneurons in the rat brain occurs in the cerebral cortex and cerebellum. The development of the cerebellum results in a progressive enlargement of the demarcate hilus and stratum granulosum (granule) which is comprised of granule cells. The total number of cells labeled by 3H-thymidine (3H-T) at various ages and sacrificing at 72 days. The time courses of GC birthdays at ventral and dorsal levels of the SG were determined by following the radioactively labeled cell population. Unilaterally the time course for GC birthdays was unaffected. Dorsally, GC birthdays were significantly depressed on day 5 probably reflecting the prolonged inhibition of cell proliferation in this region. This fall was in marked contrast to GC genesis in control which was maximal on day 5 and resulted in a late peak in GC birthdays at the ventral level which was found. The time courses of GC birthdays at ventral and dorsal levels of the SG were determined by plotting the ratio of heavily labeled GC/total GC versus age. Integrated area measurements in serial sections showed that the total volume of the SG was reduced by 20% in treated rats at 7 days, but was controlled at 60 days. These observations of decreased cell proliferation and delay in the peak of GC birthdays during HC treatment indicate that the rate and pattern of cell proliferation in the SG are altered by hydrocortisone treatment.
The effects of protein-calorie malnutrition on the brain are one of the major problems of our time. Malnutrition in animals during early life has been found to cause anomalies in the development of neuronal systems, reduction in cell size, neuronal cell bodies and other abnormalities. Children who have endured severe malnutrition in early life manifest varying degrees of retarded learning ability. Because of the critical involvement of the dendrites and the synapses in neurological function, the ultrastructure of cerebral capillaries was also studied in these animals.

The purpose of this study therefore was to determine the effect of protein malnutrition on the morphology of brain structures. Some of the experimental animals were fed isocaloric diets containing either 25% casein (well nourished) or 8% casein (malnourished) for two weeks prior to mating and throughout gestation and lactation. Diet regimens were continued after weaning in the young and subsequently the male and female offspring were sacrificed at 35, 100, and 300 days after birth and the brains were fixed for electron microscopy.

Examination of the brains showed that a significant decrease in the number of capillaries was present in the malnourished animals. Concomitant with the decreased weight was a decrement in synaptic morphology as well as a significant decrease in the number of endothelial mitochondria per cerebral capillary profile. These observations suggest that the decreased energy for active transport across the blood-brain barrier is a functional impairment at the cellular level.

The results of these studies provide both qualitative and quantitative morphological evidence of a loss of function in the brain due to protein malnutrition. Decreased brain size, loss of mitochondria, capillary atrophy and altered synaptic structure suggest a functional impairment at the cellular level.

This research was supported in part by HSU grant MHE 05792-01.

The effect of protein malnutrition on the morphology of brain structures is of great importance. For instance, undernutrition of the developing brain results in a significant decrease in the number of endothelial mitochondria per cerebral capillary profile. The loss of mitochondria and reduced energy for active transport across the blood-brain barrier suggest a functional impairment at the cellular level.

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The EFFECT OF LEAD Exposure ON DEVELOPMENT OF HIPPOCAMPAL MOSSY FIBER SYNAPSES was investigated. Jerrolynn B. Campbell*, Vijaya K. Vijayan and Dorothy E. Woolley*. Departments, Human Anatomy and Animal Physiology, Univ. Calif., Davis, CA 95616

The development of mossy fiber synapses was mapped. The results suggest that lead exposure significantly alters the development of mossy fiber synapses. These alterations are more characteristic of that found in the adult rat (unpublished data). It is possible that these changes are due to the reduced precursor availability due to the effect of lead on blood flow to these brain regions, or to the newly synthesized synapses in the developing brain.

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RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR IN CULTURED RAT SYMPATHETIC NEURONS. Robert B. Camenpest, Edward Hamroit and Paul H. Patterson. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

Nerve growth factor (NGF) is essential for the survival of sympathetic neurons in vivo and in culture. The retrograde transport of NGF to the sympathetic ganglia has been demonstrated in vivo (Hendry et al., Br. Res. 1974). We have begun an examination of the retrograde transport of NGF in rat sympathetic neurons maintained in a 3-day coculture system developed by Campenost and Edin, 1977. Neurons send their axons across a fluid-impermeable seal into 2 separate chambers located on either side of the cell body. It is quite common for a single neuron to have axons extending into both chambers. Upon incubating endings in one chamber with 125-I-NGF, radioactivity appears in the cell bodies at a time consistent with an axoplasmic transport rate of 3-5 μm/hr. An apparent steady state was reached in ~10 hrs and was maintained for at least 34 hrs continued incubation. The cell body-associated radioactivity is not due to leakage of 125-I-NGF across the seal and direct uptake by the cell bodies since incubating the cell body chamber with excess unlabelled NGF has no effect on the appearance of 125-I-NGF in the cell bodies. On the other hand, incubation of nerve endings with 125-I-NGF in the presence of excess unlabelled NGF reduces the amount of retrogradely transported radioactivity by >90%. Furthermore, transport is largely inhibited by 20 μg/ml colchicine. 35S gel electrophoresis indicates that the cell body-associated radioactivity migrates as one band with the appropriate molecular weight of NGF. Thus, these findings with isolated NGF in culture are consistent with the results obtained in vivo. Although 125-I-NGF was transported retrogradely to the cell bodies, none of this radioactivity migrated further from the cell bodies in the orthograde direction into endings located in the opposite chamber. This is consistent with the observed local effects of NGF and the transport of NGF into spinal ganglia of the chick embryo (Brunso-Bechtold and Hamburger, PNAS, 1979). Effects of NGF and the transport of NGF into spinal ganglia of the opposite chamber. This is consistent with the observed local effects of NGF and the transport of NGF into spinal ganglia of the chick embryo (Brunso-Bechtold and Hamburger, PNAS, 1979).

Cultured sympathetic neurons are able to transport 125-I-NGF when exposed to concentrations as low as ~0.5 ng/ml (2 x 10^-12M) suggesting the existence of a high affinity uptake mechanism in the nerve endings. The retrograde transport appears to be regulated by the external NGF concentration. Neurons starved of NGF for 24 hrs showed a 5-fold increase in the amount of 125-I-NGF retrogradely transported. Binding studies performed on dissociated neuronal cultures suggest that this regulation is not at the level of the surface receptor as detected by the low temperature specific activity of the receptors.

(Supported by the Helen Hay Whitney Foundation and the NINDS.)


In order to determine the degree to which central visual circuitry is dependent upon retinal input we have raised Rana pipiens tadpoles through metamorphosis after either bilateral or unilateral removal of the eye primordia. The operation is performed at Shumway stage 17 before optic nerve axons have entered the brain.

Bilateral eye removals result in a ~70% decrease in postmetamorphic tectal cell numbers compared to operated controls. Tectal layers 1 through 6 remain distinct in these developmentally uninnervated tecta, but layers 7 through 9 are condensed. Volume and laminar changes are qualitatively similar but less pronounced in the contralateral tectum. The results obtained in these experiments show an absence of significant changes in the contralateral tectum after unilateral eye primordia removal.

The nuclei projecting to these tecta have been identified in postembryonic animals using HRP histochemistry. A control analysis of normal animals agrees with those of previous authors (Wilson and Northcutt, 1977, JCN 173:219-229) in specifying the pattern of fibers that project to the diencephalon. After bilateral eye primordia removal small bundles of HRP placed beneath the dorsal tectal surface label all ipsilateral nuclei that project to that region in normal animals. Contralateral, cell groups in the diencephalon remain prominent but lose their caudal and rostral terminations. Progressive reduction of the optic tectum occurs. Thalamic nuclear cell bodies of the thalamus are a source of label in the tectum of normal animals; however, these cell populations are not maintained after bilateral eye primordia removal.

About 80% of PLC activity was abolished and the protein consisted of only one subunit (M.W. about 15000). Effects of heated β-BTX on the development of the trochlear nucleus and superior oblique muscle were examined. Either 2 or 4 μg of toxin/day was applied to the visualized choroidallantoic membrane of the white Peking duck embryo through an opening in the shell. Embryos injected daily just prior to and during the period of maximum embryonic cell death (days 11 to 18) indicate an increase of about 25% of neurons over the control in the trochlear nucleus. Injections of toxin prior to the onset of cell death (days 8-11) indicate that toxin has no stimulatory effect on cellular proliferation of the young trochlear neurons. Similarly, injections of toxin after the period of cell death (days 21-24) indicate that toxin does not cause a transient increase in the number of trochlear neurons. Thus, it appears that β-BTX increases the number of trochlear neurons when applied during the period of cell death but does not retard the magnitude of cell death. β-BTX delays differentiation of the superior oblique muscle. For example, on day 18 the control muscle is composed of myoblasts and myofibers and the motor endplates become identifiable by cholinesterase staining. The toxin-treated muscle on day 18 is composed of myotubes and myofibers. The motor endplates do not appear until day 22 posthatching.

(Supported by NIH Grant GM20348.)
ACTH4-10 ENHANCES RETENTION OF CONDITIONED TASTE AVERSION LEARN­

ing. In a series of experiments, it was found that ACTH4-10 administration enhanced the retention of taste aversion learning in rats. Specifically, rats injected with ACTH4-10 before conditioning showed a greater reduction in intake compared to control rats injected with saline. This effect was observed even when the administration of ACTH4-10 was delayed by up to 6 hours after the conditioning event. The results suggest that ACTH4-10 plays a role in the consolidation of memory for taste aversion, with potential implications for understanding the mechanisms of learning and memory consolidation.

TREATMENT WITH ACTH1-24 HAS PROLONGED EFFECTS ON PHYSIOLOGY AND DEVELOPMENT. The administration of ACTH1-24 to rats demonstrated prolonged effects on both physiology and development. Specifically, treatment with ACTH1-24 led to changes in body weight, food intake, and exploratory behavior, which persisted for up to 24 hours after administration. These effects were observed in both male and female rats, suggesting a sex-independent mechanism. The prolonged effects of ACTH1-24 may have implications for the study of neuroendocrine regulation and its impact on physical and behavioral development.

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FUNCTIONALLY DISTINCT FACTORS SUPPORTING THE SURVIVAL OF CHICK SENSORY AND SYMPATHETIC NEURONS IN CULTURE. David Edgar*, Yves Barde* and Hans Thoenen. Dept. of the conditioned media or tissue extracts. Here we dissociated chick embryo spinal ganglia. This property is able to support the survival of sensory neurons from culturing neurons from chicks of embryonic ages ranging between day 7 and day 15 in ovo revealed that the presence of NGF, as specific anti-NGF antibodies, will be discussed with reference to those showing that the responses of sympathetic neurons, distributed, the glia alone, having survived the elimination of multiple nucleoli (Multi) or a single nucleolus containing no nuclear infolding were also evident in both experimental groups, indicating further alterations in the synthetic mechanism of developing neurons exposed to ethanol. How much of the increased retinal degeneration seen in runts was directly due to ethanol and how much indirectly to malnutrition is not yet clear.

GROWTH AND DEVELOPMENT OF THE OPTIC NERVE IN JUVENILE GOLDFISH. Stephen S. Caster, Jr., Phillip E. Kish* and Steven S. Scherer*. Div. Biol. Sci.-U. Michigan, Ann Arbor, MI 48109. Previous studies have shown that the retina of juvenile goldfish grows by adding new cells, including ganglion cells, photoreceptors, and horizontal cells. It is known that the optic nerve grows in young and old goldfish and finds structural changes which accompany retinal growth. Optic nerves and tecta were prefixed in 4% glutaraldehyde for 2 h with buffer and were then postfixed in 1% OsO4 in DNP. Sections were stained with toluidine blue and examined light microscopically. Thin sections were stained with U and Pb and examined electron microscopically.

The table gives a quantitative comparison of the optic nerves of young and old fish (ages: 1 and 5 yr; standard lengths: 4 and 12 cm; weights: 47 and 87 gm; length measurements: 1.5 and 4.0 mm). Mean values in:

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In the orbit, the optic nerve is accompanied by the retinal vessels and a small nerve, containing tens of fibers, which enter the choroid. Glial processes divide the optic nerve into fascicles, the number of which varies depending on the level of section. Small groups of fibers commonly cross between fascicles. In a few cases, the fascicles were widely separated. In small fish, there is always one fascicle which contains nearly exclusively non-myelinated fibers which number a few thousand and measure a few tenths of um in diameter. These are probably the fibers from the new ganglion cells because: 1) new fibers are expected to be non-myelinated and 2) these fibers segregate to the lateral geniculate body. In older fish, the fibers appear to be more separated from the lateral geniculate body. The similarity of fibers from juvenile and old fish suggests that new fibers fasciculate near preexisting fascicles of slightly older fibers. The relative absence of non-myelinated fibers in nerves of large animals suggests that these animals' retinas have stopped adding ganglion cells.

Supported by NIH grant EY-00168.


Culture medium conditioned by C-6 glioma cells is able to support the survival of sensory neurons from dissociated dorsal-root ganglia in culture. This property is also shared by a well characterized protein, the nuclear envelope, which is present in all eukaryotic cells. This structure in neurons. Central chromatolysis and extensive nuclear infolding were also evident in both experimental groups, indicating further alterations in the synthetic mechanism of developing neurons exposed to ethanol. How much of the increased retinal degeneration seen in runts was directly due to ethanol and how much indirectly to malnutrition is not yet clear.

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When the cells of developing CNS are mechanically dissociated and planted in appropriate concentrations in cultures, cell colony development is prerequisite for the formation of a colony that is a cell must adhere to the substratum (plastic) and proliferate. We investigated the possibility of using cell colony formation as an assay to monitor the proliferative cell compartment during CNS development.

The spinal cord of chick embryos, cerebral hemispheres of mature embryos at various developmental stages, and the spinal cords of postnatal mice were isolated and the meninges were removed. The basal ganglia and hippocampus were separated from the spinal cords. These embryonic hemispheres were then dissociated into cell suspensions which were plated in various concentrations in Falcon plastic dishes. After 7, 10 and 14 days of culturing, the numbers of colonies formed as a function of the size of inocula were determined by direct counts under a light microscope.

We found that 75% of colony forming cells came from the subventricular zone of the brain. The ultrastructure of the cells resembled the epithelial type colonies was similar to that of the "pale" cells of the subventricular zone.

We describe in this study that the colony forming cells are derived primarily from the secondary proliferative zones such as the subventricular zone and that their formation in the CNS and their progression into postmigratory cells can be monitored with the aid of colony culture assay method. This work was supported by Grant MT4235 from MRC Canada.

The chick embryo spinal cords yielded only few colony forming cells up until 4.5 days (E4.5) of development. Between E5.5 and E7.5 there was an exponential increase in the yield of colony forming cells; the peak yield was reached at E11.5. Postnatally, the yield began to decrease gradually and at P30 few colony forming cells were visible. In another series of experiments the mouse cerebral hemispheres were cut into small portions which were divided into two fractions: An inner fragment containing the cortical plate and most of the intermediate zone, and an inner fragment containing a small portion of the intermediate zone, the subventricular zone and the ependymal layer. Such fragments were dissociated and plated in culture dishes. It was found that 75% of colony forming cells came from the subventricular zone of the brain. The ultrastructure of the cells resembled the epithelial type colonies was similar to that of the "pale" cells of the subventricular zone.

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MATERNAL USE OF ALCOHOL, CIGARETTES AND/OR MARIJUANA DURING PREGNANCY: EFFECTS UPON THE OFFSPRING. Peter A. Price* (SPON: M.N. Blaby) Lions Sight Centre, Division of Morphological Science, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

A sample of 200 predominantly middle class women were interviewed once during each trimester and questioned about their use of alcohol, cigarettes and marijuana. The babies of a number of these women were examined at birth, 2, 9 and 30 days of age on a variety of tests that included assessment of behavioral and neurological development. A reduction in birth weight was observed in babies born to mothers who smoked more than 10 cigarettes a day or drank an average of just over a drink or more a day or smoked more than 5 "joints" of marijuana per week. Cigarettes had the most marked effect and a combination of these soft drugs did not greatly potentiate the reduction in birth weight. Cigarettes, and to a lesser degree, social use of alcohol were related to decreased head circumference in the newborn. A decrease in responsiveness to auditory stimuli was found in the offspring of smokers and habituation rates were slower among babies born to smoking marijuana users. A very pronounced increase in irritability was observed in the offspring of marijuana users and, to a lesser degree, in the babies of heavy social smokers. A number of pigs (both related to marijuana usage during pregnancy and to a slightly lesser extent were also found in the offspring of heavy social drinkers and/or smokers. The relationship between maternal use of soft drugs and subtle neurological abnormalities in offspring are discussed.

QUANTITATIVE ANALYSIS OF NEURONAL DEVELOPMENT IN THE GUINEA PIG RETINA. Keith R. Fry* and Arthur W. Spira* (SPON: M.A. Bisby)

Differentiation of many of the main components of the guinea pig retina e.g. synapses, outer segments appear well advanced at birth compared to many other species (Spira, Anat. Embryol. 146: 146-279, 1975). How do populations of neurons change throughout development and when do these populations achieve full differentiation? Quantitative light microscopic techniques have been employed to study neuronal development in the guinea pig retina. Retinal thickness is recognized to play a major role in the development of retinal morphology, location within the inner nuclear layer, thickness and the cytoplasmic/nuclear ratio to one of the following categories: (1) horizontal (I) amacrine (ii) rod or cone bipolar (iv) undifferentiated and Müller. Number of each type of cell varies with a logarithmic growth curve throughout the life of the animal. Many of these cells can be characterized by distinct connections to other retinal layers. The hypothesis that connections between retinal and tectal cells are temporarily formed and later broken. Their hypothesis, further states that the in the newborn. A decrease in responsiveness to auditory stimuli was found in the offspring of smokers and habituation rates were slower among babies born to smoking marijuana users. A very pronounced increase in irritability was observed in the offspring of marijuana users and, to a lesser degree, in the babies of heavy social smokers. A number of pigs (both related to marijuana usage during pregnancy and to a slightly lesser extent were also found in the offspring of heavy social drinkers and/or smokers. The relationship between maternal use of soft drugs and subtle neurological abnormalities in offspring are discussed.


An ordered projection of retinal ganglion fibers can be found on the tectal surface throughout most of Xenopus larval life. The presence of this ordered retinotopic projection has been taken to imply that ordered retinotectal connections are formed during ontogeny. However, since there are asymmetries in the development of the retina and tectum, Cazal et al. have proposed that connections between retinal and tectal cells are temporarily formed and later broken. Their hypothesis is further supported by the observation that during the growth of the eye and tectal cortex there is a continuous caudal migration of synaptic contacts between retinal ganglion cells and tectal cells. In the present study, I have isolated visually driven post-synaptic tectal unit activity in larval Xenopus laevis. Utilizing standard electrophysiological techniques, post-synaptic visually driven units were isolated and characterized first in the rostral-lateral tectum of adult and juvenile Xenopus laevis. These cells, found in the deep tectal layers, respond to larger stimulus targets within larger visual receptive fields than did retinal ganglion terminals. Post-synaptic units, unlike presynaptic fibers, also habituated to the successive repetition of a visual stimulus. Furthermore, the post-synaptic response could be eliminated by the topical application of curare or sodium pentobarbital, whereas presynaptic activity was unaffected by these drugs. Visually driven retinal fiber terminals were found in the rostral-lateral tectum of the youngest animals studied (stage 49). However, on the basis of criteria established in the adult and juvenile preparations, post-synaptic units could be isolated earlier than stage 52-53. The implications of these findings will be discussed.
ORIGIN, TRANSFORMATION, AND DEATH OF NEURONS FROM AN IDENTIFIED PRECURSOR DURING GRASSHOPPER EMBRYONIC DEVELOPMENT. E. Bate*, and N.C. Spitzer. Dept. of Biology, UCSD, La Jolla, CA 92037, and Max-Planck-Institut für Virosvorschung, Tübingen, DDR.

We are interested in the differentiation of identified neurons from their birth to their maturation in the grasshopper embryonic development. We have reported that an identified neural stem cell gives rise to identified neurons, and described their morphological, physiological, and biological differences. Here we report on the differentiation of neurons that are the progeny of a different class of precursor cells. There are seven of these cells per segment, called midline precursors (MP 1-7), which are the anterior and posterior processes of the unpaired median (DUM) neural stem cell whose progeny were the subjects of our previous study.

The progeny of the single division of cell MP 3 were examined by serial section reconstructions of light microscopic sections, direct observation of cells with interference contrast optics, and intracellularly injected horseradish peroxidase. Within a few days thereafter the first unilateral process disappeared and the cell assumed the unpaired, bilateral symmetric identified "H" neuron. Its label 65% died at this time; when it persists it retains only its original unilateral process.

During the course of embryogenesis, the soma of the "H" cell assumes a position immediately anterior to the older progeny of the MP neural stem cell. Thus we have been able to follow the origin and transformation of a single cell which seems to assume two different roles during embryogenesis. It first appears as one of a pair of putative "pioneer" fibers and second transforms into an unpaired identified neuron.

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The rotation of embryonic eyes is a classic experimental procedure in studying retino-tectal connections in amphibian. The optic fiber (OF) pathways that developed after such operations, however, have not been adequately studied. Conceivably, the manner whereby OF return to the tectum may play an important role in retino-tectal wiring. Whether the optic fiber pathways that develop are dependent upon local environmental conditions encountered by growing OF as they emerge from a rotated eye or are determined by the local age of retino-tectal innervation of the eye, we rotated Xenopus eyes at embryonic stages 21/22 to 23 and traced the OF pathways by prolne radiograph in mid tadpole stages.

The main findings include: (1) optic fibers project caudally along the descending tract of the trigemino in the spinal cord, to the brain and to the terminal abdominal ganglion. The number of such cells decreases during the prenatal period, and few were seen by day 18. These cells may be migrating motoneurons. The fetal motoneurons appeared immature in that they often had dendrites extending into the ventral white matter and lacked the longitudinally direct dendrites characteristic of more mature motoneurons.

At 14 days of age, the dorsal root ganglion cells were filled with antigens at day 14, indicating the presence of peroxidase in the periphery. Anterior fibers with clearly identifiable growth cones were observed in fetal spinal cord cells from the 15-18th day. Anterior fibers grew longer than axons of the same species, which remain in the periphery. The axons in the peripheral nervous system (PNS) have their major arborizations in the restricted regions to which they project in rats 10 days after birth.

We have previously shown that extensive tubulin microheterogeneity is prominent in the brain, whereas tubulin isolated from other organs is less heterogeneous. Mature rat brain tubulin can be resolved by isoelectric focusing into nine distinct components. Different proportions of these nine isotubulins are displayed by various brain regions, resulting probably from the heterogeneity of precursor cell populations. The enrichment in tubulin heterogeneity is developmentally determined, increasing from five to six components (isotubulins 1-6) in the prenatral rat brain to nine components (isotubulins 1-9) in the mature brain (isotubulins 1-9).

We have previously shown that extensive tubulin microheterogeneity is controlled at the RNA level, results from post-transcriptional modifications. In the present study, mature brain mRNA was translated in vitro in the reticulocyte lysate cell-free system, and was found to reflect the synthesis of several tubulin isotypes. The mRNA species directing synthesis of tubulins 1, 3, 4, 5, and 6 showed differences in mobility. It therefore appears that brain mRNA may consist of five different species coding for distinct tubulin forms. Moreover, age-dependent changes in the relative translation of the mRNA coding for isotubulin 7, which is not evident at the translation stage, were found at the mRNA level.

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510 THE DEVELOPMENT OF THE CORTICAL COLUMN: A (+4C)-2-DEXTROGLUCOSE STUDY IN THE RAT. F. Hand, M. Kosaut*, U. Patel*, and C. Goodspeed.* Dept. of Radiation Biology, School of Veterinary Medicine, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania, 19104; Department of Neurophysiology, Rockefeller Institute, Experimental Pathology, Warsaw, Poland; and Laboratory of Cerebral Metabolism, NIMH, Bethesda, Maryland 20014.

The dextrose-2,3-O-6 (2DG) studies of Hand et al., (Neurosci. Abst. 1973:77) demonstrated that staining of a fraction of the vibrissae increased glucose utilization in a single, spindle-shaped cortical column which extended from the superficial to the deep layers of lamina VI. The precision of this system makes it a useful model in which to investigate plasticity. Hand et al., produced significant increases in labeling in the singular vibrissae after unilateral ablation of all but one vibrissal column (C3), 2-4 days postnatally (P-2, P-4). The column activated by C3 stimulation was densely labeled only on days 10-12. In contrast, the diffuse labeling of supra- and infragranular layers was the result of a reorganization of developing axonal terminations or the development of neurotransmitter function was unaffected by the selective columnal lesion. Large numbers of pyknotic cells were observed in the thoracic and lumbosacral spinal cord (T/L) in the third group. The occurrence of intramedullary hemorrhage and necrosis was not significantly different. These results indicate that the Purkinje cell population of the differentiating rat cerebellum is susceptible to the actions of centrally active agents during the period of neuronal differentiation, both pre- and postnatally. (Supported by Alberta Mental Health).


It is currently believed that many therapeutic agents which can cross the placenta may produce subtle effects on the central nervous system of the developing fetus and during the lactation period postnatally, on the neonate. It is our hypothesis that centrally active drugs effect networks which are susceptible in neuronal population resulting from the action of these drugs on the differentiating neuronal membranes, thus preventing the formation of central synapses. The two drugs chosen for this study were Chlorpromazine and Phenoobarbital. Both agents are capable of crossing all membrane barriers and are frequently administered both pre- and postnatally. Chlorpromazine was chosen as a prototype of the pheno-thixine tranquilizers and antiepileptics and pheno-barbital as a prototype of the barbiturates mediating hypnosis. Chlorpromazine and Pheno-barbital groups of time-pregnant Sprague-Dawley rats were given daily I.P. injections of either 0.6 mg/kg Chlorpromazine or 0.4 mg/kg Pheno-barbital on postnatal days 3, 5, 7 and 9. These results indicated that the pheno-barbital cell population of the differentiating rat cerebellum is susceptible to the actions of centrally active agents during the period of neuronal differentiation, both pre- and postnatally. (Supported by National Institutes of Health).

520 EFFECTS OF CELL-SUBSTRATE INTERACTIONS ON THE SURVIVAL AND DEVELOPMENT OF CULTURED SYMPATHETIC NEURONS. Edward J. Hawrot* (SPON: Story Landis). Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

The maintenance of low-density, dissociated rat sympathetic neurons for long times in culture requires strong adherence of the extending neurites to the culture substrate. Several chemically-differentiated surfaces were selected as well as some cell-derived or of newly established connections (sprouting) in P-4 rats. We postulate that lack of labeling in cortical laminae VI and V, containing neurons projecting to thalamus and brainstem-spinal cord, may be responsible for the lack of cortical control in neonates. (Supported by grants NS-06716 of USPHS and 76-10-9 of NIH, Canada).
DEVELOPMENT


It has frequently been suggested that before axonal outgrowth sensory neurons are unspecified and that they are later specified by contact with their peripheral targets. Alternatively they may somehow be intrinsically specified. The aim of our experiments has been to determine whether any such differences exist and in addition the pattern of initial afferent outgrowth has been examined to determine whether it is selective or random.

Although sensory neurons are projecting out peripheral targets, the medial dendrites of the deprived int-1 cross the midline of the contralateral auditory neuropile on the contralateral side. Moreover, morphological development in the cricket Teleogryllus oceanicus, its medial dendrites grow in a peculiar pattern. Normally, the medial dendrites project to the ipsilateral acoustic neuropile and never across the midline. We conclude that deprived interneuron-ls (Hoy, Casaday, & Rollins (1978) Soc. Neurosci. Abst. 4.) are innervated by contralateral auditory afferents.

INTERNEURON ARE INNERVATED BY CONTRALATERAL AUDITORY AFFERENTS. Ronald Hoy and Andrew Moiseff. Sect, of Neurobiology & Behavior Langmuir Laboratory, Cornell University, Ithaca, N.Y. 14850.

Hoy, in prep.) .

Unilaterally deprived preparations suggest that in unilaterally deprived animals the intact auditory neuropile on the operated side retains the normal. In the intact ear on the contralateral side, deprivation of the auditory stimulus is associated with a reduction in the number of auditory neurons in the operated side. This result is consistent with the hypothesis that low intensity sensory neurons are more likely to be specified to innervate their appropriate targets. (Supported by NIH NS10666).

525 ABERRANT DENDRITIC PROJECTIONS FROM AN IDENTIFIED AUDITORY INTERNEURON ARE INNERVATED BY CONTRALATERAL AUDITORY AFFERENTS. Ronald Hoy and Andrew Moiseff. Sect. of Neurobiology & Behavior Langmuir Laboratory, Cornell University, Ithaca, N.Y. 14850.

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Unilaterally deprived preparations were physiologically tested to determine whether the deprived interneuron-ls could be activated by acoustic stimulation of the intact ear on the contralateral side. We were able to record units from both the deprived and intact sides that responded to both the deprived and intact ears. These results suggest that localized afferent inputs to the deprived interneuron-ls can be activated by acoustic stimulation of the intact ear. This finding is consistent with the hypothesis that at least some sensory neurons may be pre-specified to innervate their appropriate targets. (Supported by NIH NS10666).


Studies employing catecholamine histochemistry have shown that adrenergic axons first appear on the 11th incubation day in the chick embryo right ventricle (RV). We have studied the functional properties of the membranes of the pre-synaptic adrenergic axon. The post-synaptic cardiac muscle, however, has shown a delay in the appearance of synaptic formation. Terminals of developing adrenergic axons were characterized by their ability to transport and retain tritiated norepinephrine ([H]-NE) and to secrete previously labeled NE. RV were excised from chicks (9 days after fertilization) and incubated for 1 hour in Tyrode solution containing [H]-NE (0.5 µM). They were then transferred to a chamber where passive overflow of [H] was monitored for 60 minutes. Subsequently release was evoked by elevated potassium (K*, 140 mM) or by 60 seconds of electrical excitation (ES) of intracardiac nerves (50 pulses, 5 msec, 30 Hz).

Cocaine-sensitive uptake and reserpine-sensitive retention of [H]-NE were first seen during the third embryonic week. Release of [H] by K* or ES was seen as early as the 14th embryonic day. The amount of [H] released per mg tissue by either method increased between 30 days and 40 days of age.

20 min neonatal anoxia did not differ in avoidance responding compared to controls. However, neonatal rats subjected to 6% O2 for 4 h but not in the adults are markedly inferior in the CAR acquisition than the control group. The amount of [H] released per mg tissue by either method increased between 30 days and 40 days of age. Neural crest migration . This reverses both the prospective DRG and the spinal cord. Displaced motoneurons form appropriate connections in this situation. (Lance Jones and Landgren, 1978, Neuroni, Abst. 4;118.) For sensory neurons the pattern of outgrowth is not completely random and projections down appropriate nerves can sometimes be found from displaced ganglia. These results are consistent with the hypothesis that at least some sensory neurons may be pre-specified to innervate their appropriate targets. (Supported by NIH NS10666).


In the rat brain, almost all the catecholamines-C containing nerve cell bodies present in the adult are also present at birth. The rate limiting step in the formation of the neurotransmitters dopamine (DA) and noradrenaline (NA), tyrosine hydroxylase is markedly affect­ed during oxygen deprivation, as molecular O2 serves as a substrate for the synthesis of the intermediate substance dihydroxyphenylalanine (DOPA). Neonatal rats were exposed to a low oxygen environment (anoxia or 6% O2) for various time intervals. DOPA accumulation was measured after N S 1015 to estimate the rate of tyrosine hydroxylation. In vivo and DA and NA levels were followed after α-methyltyrosine to study the nerve impulse activity. In the CA neurons. During a short period of hypoxia (6% O2, 20 min) the synthesis of DA and NA was impaired in the brain stem, "midbrain" and striatum regions of the neo­natal rats. 6% O2 over a 2 h interval also caused a reduced nerve impulse activity in the DA neurons but not in the NA neurons, as measured by DA and NA disappearance after α-methyltyrosine. The accumulation of a conditioned avoidance response (CAR) and the effects on CA neurotransmitter synthesis and levels were investigated in 28 days old rats after neonatal oxygen deprivation. A group of rats exposed to 20 min neonatal anoxia did not differ in avoidance responding compared to controls. However, neonatal rats subjected to 6% O2 for 4.5 h were markedly inferior in the CAR acquisition than the control group. CA synthesis, measured as DOPA accumulation after N S 1015, was impaired in the rats exposed to 6% O2 for 4 h but not in the an­imals exposed to 8% O2 for 20 min before concluded that the periods of acute asphyxia markedly affect CA synthesis and turnover in the neonatal rat brain. It is also suggested that the specific beh­avioral deficits observed after prolonged neonatal asphyxia may be due to an impaired development of central catecholamine mechanisms.
DEVELOPMENT


Chicks at the four ages: 10 days in ovo (i.o.), 1 day and 30 days after hatching (a.h.) and 10 days a.h. were fixed by perfusion and the lumbar paravertebral ganglia, Li-L4, were prepared for electron microscopy. The volume of each whole ganglion was calculated from areas measured on 200 µm thick sections. Partial volumes of neuronal somas, neuropil, connectives and blood vessels were determined by point hit counting of 200 µm thick sections taken at random intervals from thick sections. The absolute volume per ganglion of each compartment was determined as the product of the average volume per ganglion from serial sections and the partial volume of the compartment. Preliminary results are presented here.

The partial volume of the neuropil increases with age from less than 10% at 10 days i.o. to 35% at 10 days a.h. The increase in greatest in the interval before hatching and is substantially less, consequently. By contrast, the partial volume of neuropil in 10 day is less than 10% and decreases progressively to about 20% in the adult. Total ganglionic volume increases progressively from 0.01 mm³ per ganglion to 0.11 mm³ in the adult. The increase in their absolute volume per ganglion. The absolute volume of neuropil per ganglion increases during each of the three intervals studied. The ratio of the absolute volume of neuropil to absolute volume of somas also increases substantially during each interval, from 0.3 at 10 days i.o. to 2 in the adult.

Using the radiochemical microtechniques of McCaman and Stettler (J. Neurochem. 19:411-416, 1972) the labeled choline (ACH) and choline were also measured in chick lumbar sympathetic ganglia. Data are expressed as pmol/ganglion. ACH increases over 7 fold (6.6) between 0-1 day i.o. to almost 8 fold levels are low (3.0) throughout embryonic and early posthatching stages and rise rapidly between 7 days a.h. and adult. Choline levels closely parallel those of ACH and increase during this period. Although a general correlation between the amounts of ACH and of choline at different ages, the patterns differ in detail. These differences may reflect changes with age in the ultrastructural composition of the neuropil, currently under analysis. Our results show that growth and development of these ganglia continues well into adulthood. Growth is evidenced by substantial increases (at least doubling) in the total ganglionic volume, the absolute volume of neuropil per ganglion, and in the absolute volumes of ACH and Choline per ganglion. In the period between 30 days and 360 days a.h. (Supported by grants NIMH 5 F31 MH07326, NS09904 and UCORNS Res. Found.)


The egg of Aplysia is determinate: specific cells of the blastula are recognizable as neurons. We are also investigating the migration of the neuroblasts and initiate their differentiation following final division and 3) the three-dimensional arrangement of the neurons within the ganglion.

Exposing egg masses in the late trocophore stage (6&7 days after fertilization, respectively) resulted in the labeling of fewer cells. At larval stages and rise rapidly between 7 days a.h. and adult. Choline levels closely parallel those of ACH and increase during this period. Although a general correlation between the amounts of ACH and of choline at different ages, the patterns differ in detail. These differences may reflect changes with age in the ultrastructural composition of the neuropil, currently under analysis. Our results show that growth and development of these ganglia continues well into adulthood. Growth is evidenced by substantial increases (at least doubling) in the total ganglionic volume, the absolute volume of neuropil per ganglion, and in the absolute volumes of ACH and Choline per ganglion. In the period between 30 days and 360 days a.h. (Supported by grants NIMH 5 F31 MH07326, NS09904 and UCORNS Res. Found.)

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The developing cerebellar cortex provides an ideal system to evaluate the change in radiosensitivity that occurs in the course of differentiation of granule cells. The proliferative, migratory, and maturation stages of granule cells are present concurrently in anatomically discrete layers and can be histologically quantified.

Ten day old Mistar rat pups were given a single dose of 3000 KV whole head x-rays ranging from 25 to 7000 r. The pups were sacrificed 6 hrs after irradiation and the cerebellum were embedded in glycol metheracrylate. Coronal sections were cut at 3 microns and stained with hematoxylin. All cells were counted in samples of the external germinal layer (EGL), molecular layer (ML), Purkinje cell layer (PCL), and internal granule cell layer (IGL) of the pyramids. The relative reduction of each cell type across dose was then determined.

Mitotic activity ceased above 250 r. The germinal cells of the upper portion of the EGL and the premigratory cells of the lower portion of the EGL were reduced by 80% at 300 r. The descending granule cells of the ML and PCL were not significantly reduced until a dose of 1000 r. The basket cells of the ML did not exhibit significant pyknosis until a dose of 7000 r. Purkinje, Golgi and Bergmann glial cells did not exhibit significant pyknosis at the doses applied. With the exception of the small contribution of the basket cells, the entire pyknotic population can be accounted for by the selective elimination of granule cells at different stages of their differentiation.

The decrease in radiosensitivity occurring with differentiation and associated migration may reflect functional metabolic or morphological specification. The selective elimination of undifferentiated granule cells can be used to determine their effect on the development of the cells with which they form connections as well as the circuitry in which they are normally involved.

**References**


They increase in number over the ensuing 24 hr. period, but by 13.5 days these noradrenergic characters have essentially disappeared. To define the fate of these cells, we studied formaldehyde-induced fluorescence following specific, high-affinity uptake of norepinephrine (NE). NE uptake, which was apparent in gut neuroblasts at 12.5 days, persisted at least through 17.5 days, long after the other endogenous noradrenergic characters had disappeared. Uptake was inhibited by incubation at 4°C or by inclusion of the highly specific uptake blocker, desmethylimipramine. These observations suggest that (a) high-affinity NE uptake develops as an additional noradrenergic characteristic in these gut cells, (b) the ability to take up NE persists even after the disappearance of other noradrenergic traits, and (c) the loss of noradrenergic characters is probably not simply due to death of the neuroblasts. Consequently, during embryonic development neuroblasts may transiently express transmitter phenotypes.

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Pioneer neurons (Bata, '76, Nature 260:54-56) are monopolar afferents found in the lumen of insect embryonic appendages. They serve sensory functions to the central nervous system. The origin of these cells was determined in cultured embryos of the grasshopper Schistocerca gregaria undergoing normal metamorphosis. The development of the metameric leg and antenna was observed with time-lapse video microscopy using differential interference contrast optics, at 30°C. At the 25-30% stage of development (Bentley et al., J. Emb. Exp. Morph., in press), large (25-30µ) spherical cells can be distinguished in the lumen of the metathoracic leg. These cells give rise to pioneer neurons. The pioneer mother cells (PMCs) have been observed to remain in interphase for as long as six hours before undergoing cell division, then ingrowing axons. PMCs sometimes extend and retract 3-5µ day by day, and can undergo small rotations (10-20°). Mitosis of the PMCs occurs in approximately one hour.

PMCs daughter cells were identified as pioneer neurons by (1) their location within the lumen, (2) their size, (3) their monopolar morphology, and (4) by a centrally coursing axon. They usually remain quiescent for 30 to 60 minutes following mitosis, after which they begin to sprout fine digitated processes. Although these processes may extend tens of microns, they are often retracted abruptly, followed by a new extension in a different direction. Following this "exploration" of the immediate cellular environment, usually one daughter will migrate to an aepithelial position along the lumen wall, then towards the tip of the leg, and then into the middle of the lumen; finally the cell directed a projection toward the central nervous system which became an axon that grew at least 100-150µ in two hours. From the 25-30% stage of development onwards some of the PMCs can be identified in the metathoracic leg: they occur in fixed number and position in the distal portion of the limb. The PMCs have not been observed to arise from the epidermis, as sensory neurons suggest. Since the evidence suggests that they may be generated centrally and accompany the outgrowth of the appendage.


In order to examine the distribution of motoneuron axons in the chick hindlimb between the time of their initial outgrowth (St 23-24) and the establishment of specific anatomic connections (St 27), injections of HRP were made into individual segments of the lumbarosacral lomeral motor column. This procedure allowed the visualization of HRP reaction product in the motoneuron cell bodies of the following axons: at St 23-24, the spinal nerves projected only to the base of the limb bud, yet clearly were beginning to form distinct cutaneous and sciatic trunks. Labelling accompanied projection from the cranial, rostral, and caudal motor nerves, and their respective a-p axonal position is as the base of the limb. No evidence of diffuse outgrowth within the plexus region or limb tissue was found at this or subsequent stages. At the cleavage of the musculature and the motoneuron cell death period, labelled axons projected via specific anatomical pathways through the plexus to appropriate regions of the muscles. These results suggest that a period of diffuse or random axonal outgrowth followed by the death of inappropriately projecting neurons is not involved in the establishment of specific connectivity in the chick hindlimb. Similar characterization of axonal pathways at St 27-30 revealed that axons to an individual muscle tended to course in discrete tracts within the plexus and nerve trunks although they could also cross to adjacent segments.

These results, although showing that axonal outgrowth is highly selective, did not allow us to distinguish between possible mechanisms involved in pioneer neuron selection. In order to gain information about these mechanisms, transplanted optic axons were studied in spinal cords in reverse rostro-caudal orientation -- 5 grew rostrally and 1 grew caudally. The other 4 M' axons entered and grew caudally from donor hindbrains attached to host diencephalon or forebrain. 9 of the 10 M' axons grew more than 100µ rostrally in the host CNS, and all 9 followed an essentially identical ipsilateral ventral CNS route (Fig. 2), extending from the developing central thalamic region down the developing spinal cord. M' axons always grew along the same discrete route in the basal region, and thus the guidance cues which they followed must be associated with a sub-plate pathway. Normally, the MF may follow this basal substrate pathway.

A substrate pathway is a set of similar guidance cues aligned in a discrete route. Previously Katz and Lasek, 1979 (JCN 135:817) used transplanted optic axons to demonstrate a substrate pathway running through the rostral region of the developing nervous system in Xenopus (Fig. 1). This alar substrate pathway has also been demonstrated in Rana Constantine-Paton, 1978 (Br Res 158:51). Here we report that transplanted Mauthner axons have demonstrated a basal substrate pathway. Extra hindbrains were transplanted to various locations in the neural tubes of Xenopus embryos at the same stages. Tadpoles were examined in 10 um Bodian stained paraffin sections at St 43-47. Among the tadpoles studied we found 10 donor Mauthner axons (M' axons) entering host CNS, 6 of these entered from donor hindbrains implanted in spinal cords in reverse rostro-caudal orientation -- 5 grew rostrally and 1 grew caudally. The other 4 M' axons entered and grew caudally from donor hindbrains attached to host diencephalon or forebrain. 9 of the 10 M' axons grew more than 100µ rostrally in the host CNS, and all 9 followed an essentially identical ipsilateral ventral CNS route (Fig. 2), extending from the developing central thalamic region down the developing spinal cord. M' axons always grew along the same discrete route in the basal region, and thus the guidance cues which they followed must be associated with a sub-plate pathway. Normally, the MF may follow this basal substrate pathway.
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Fisher. Neuroscience Program and Dept. of Anatomy,

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ges in the nuclear density of the INL. Furthermore, our experiments confirm Hollyfield's

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rump lengths (CRL) of 7-25 mm (approximately embryonic days 10-18) and


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it is mimics the lateral-to-medial gradient of cell production and  

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Physical and regional differences in transport of label are no longer  

nuclear densities of layer V and VI. By PM 4, label in the lateral parietal cortex extends through the  

diastic adhesions between apposed astrocytic processes were marked,

Histochemical localization of HRP. Light microscopic (LM) examination of 8-10 µm thick longitudinal sections of the nerves of E5, E14 and P3 animals were  

injection of saline containing 2% HRP, the animals were fixed and

and/or vascular and neuronal elements. As a basis for later study of  

of horseradish peroxidase (HRP) from the vitreous into the extra­

yond the lamina cribrosa of the optic nerve that limits the spread  

mence of a transverse network of glial contacts that might form a  

agnes a function of uptake of HRP by glial  

our results suggest that the barrier to diffusion of HRP in fetal and  

s the fetal brain prior to the  

at least 5 months the host animal was perfused with Bouin's or formalin fixative, and adjacent sections of the host brain were stained with cresyl violet and KI/O'Brien-Barreau stains and the Holm's silver method. Piec­

embryonic cerebellar anlage from all gestational stages survived and  

continued to differentiate within the ectopic site. Individual specimens were  

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LOSS OF GROWTH HORMONE SENSITIVITY IN BRAIN AND LIVER DURING MATERNAL DEPRIVATION IN RATS. C. M. Ruhn and S. M. Schambos.* Department of Neuroscience, University Medical Center, Durham, N.C. U.S.A.

We have shown previously that maternal deprivation (MD) results in a decrease in brain and heart ornithine decarboxylase (ODC) activity which may be mediated by a specific suppression of growth hormone (GH) secretion. To investigate further the relationship between the effects of maternal deprivation and the regulation of tissue development by GH, we examined the ability of GH to stimulate ODC activity in maternally deprived rats. pups were removed from the mother and placed in an incubator for 2 hours, injected with GH (50 µg, s.c.) and liver ODC activity was determined 6 hours later. Littermates controls left with the mother were similarly treated with GH or vehicle. In a separate experiment maternal deprived and control pups were injected intraceresternally with GH (100 µg) and brain ODC activity was determined 4 hours later. ODC activity was significantly lower in liver and brain of deprived pups and was not stimulated by GH, while ODC activity in both brain and liver of control pups increased significantly after administration of GH. When pups that had been maternally deprived for 2 hours were returned to the mother for 2 hours and then injected with GH, significant stimulation of ODC activity in liver was observed. ODC activity did not increase following GH administration in livers of pups placed with a mother whose nipples had been ligated.

To determine if the loss of tissue response to GH resulted from a specific suppression of GH action, maternally deprived pups were treated with several other inducers of ODC activity. Ovine placental lactogen (100,1000 µg) did not stimulate ODC activity in livers of deprived pups, but PGE-1 (50 µg), dibutyryl cAMP (50 µg), and dexamethasone (0.2 µg) significantly stimulated ODC activity in both maternally deprived and control pups. ODC activity in brains of maternally deprived pups increased significantly following intraocular administration of dibutyryl cAMP (50 µg).

These findings suggest that maternal deprivation is associated with a specific suppression of tissue response to the growth promoting peptide hormone GH and that this suppression is rapidly reversed when pups are returned to the mother, and appears to be triggered by the removal of active mothering behavior, not by nutritional deprivation or the removal of the sensitizing stimulus associated with the mother. (Supported by NIH grants MH-13603 and MI-06499).

RESPONSE OF THE CAT DORSAL LATERAL GENICULATE NUCLEUS TO INFANT VISUAL CORTEX LESIONS. Douglas R. Labar* Nancy Berman, E. Hazel Murphy, Department of Anatomy and Department of Physiology/Biochemistry, Medical College of Pennsylvania, Philadelphia, PA 19129.

Various observations indicate that following infant visual cortex lesions in cats, considerable shrinkage of the dorsal lateral geniculate nucleus (LGD) occurs, and the severely shrunken nucleus contains a sparse population of surviving isolated large cells. We looked at the time course of the occurrence of these phenomena during the first few post-operative days. Eight kittens were given unilateral visual cortex lesions within 24 hours after birth. Following post-operative intervals ranging from 1 to 9 days, subjects were given a unilateral intracarotid injection of H2-proline, and brains were processed for autoradiography.

Volumes of lesioned and control LGD's were determined from projection drawings. Volumetric changes that occurred in a lesioned LGD were determined relative to the volume of the control LGD of the contralateral thalamus. At two days post-operative, the ratio of lesioned LGD volume to control LGD volume was .92; at four days post-operative, this ratio was .82; at six days post-operative, this ratio was .51; at eight days post-operative, this ratio was .40. These findings indicate a linear shrinkage of the LGD with increasing survival intervals over the period studied. Comparisons of volumes of individual autoradiographically demarcated laminae in lesioned and control LGD's showed that shrinkage is not selective for a specific lamina, but that all laminae undergo equal shrinkage, in parallel with the shrinkage at the entire LGD.

Isolated large cells in the first stage of lesioned LGD four days after the lesion. At this time they are located in the A/A1 and A1/C interlaminar zones; at the same time, similar large cells are first seen within the cellular laminae of the control LGD at this same time.

The similarity in time of appearance and location of large cells in the normal cat LGD and the isolated large cells present in that nucleus after infant visual cortex lesions suggests that these two populations represent a single class of geniculate neuron. Perhaps the normally large Y cells are able to survive infant cortex lesions.

Supported by NIH Grants EY02448, MH31268, EY20880 and NSF/BHS 7724925.

DOES CELL POSITION DETERMINE RECEPTIVE FIELD PROPERTIES OF NEURONS IN THE MOUSE VISUAL CORTEX? Vance Lemmon* and Alan L. Goodson, University of Wisconsin, Dept. of Psychology, Madison, WI 53706.

DURING DEVELOPMENT IN RATS. Edward D. Levin* and Robert E. Cowan, University of Wisconsin, Dept. of Psychology, Madison, WI 53706.

During the normal development of the neocortex, the first cells to be produced take positions in deep cortical layers, and later cells are positioned more superficially. Many studies have shown that afferents to the LGD from different sites terminate in specific laminae, that individual laminae have specific afferent targets, and that neurons in the visual cortex within any lamina have distinctive receptive field (RF) properties. How important, then, is the laminar position of the various cortical neurons in determining their afferent and efferent connections? This problem has been studied in the reeler mutant mouse, where the positions of cortical cells are grossly disturbed; neurons that are normally located in deep cortical laminae are primarily located superficially in reeler, and vice versa.

We studied the receptive field properties of cortico-tectal (CT) cells because they are located in a single cortical lamina (layer V) in normal mice but are distributed widely in the superficial aspect of reeler cortex. In addition, CT cells have distinctive receptive field properties and properties can be positively identified by antidromic stimulation with electrodes in the superior colliculus. The properties of CT cells that we studied quantitatively in normal mice and reeler mutants included rates of spontaneous activity, RF size, velocity sensitivity and spatial summation. Statistical analysis of these properties demonstrated no significant differences between CT cells in normal and reeler. These findings indicate that the detailed connections underlying the RF properties of CT cells are properly established despite their abnormal cortical location. (Supported by NIH Grants KOI-EY00621 and TOI-EY00902).

THE BEHAVIORAL TOXICOLOGY OF LOW CHRONIC DOSES OF HALOTHANE DURING DEVELOPMENT IN RATS. Edward D. Levin* and Robert E. Cowan, University of Wisconsin, Dept. of Psychology, Madison, WI 53706.

Chronic, low level exposure of rats during development to the anesthetic halothane has been shown to cause deficits in shock-motivated spatial learning, food-motivated spatial learning and shock sensitivity. (Dubov, Katz and Rowan, Anesthesia and Analgesia, 54(5):629-33, 1975). We exposed Sprague-Dawley female rats to 12.5 parts per million of halothane in air for 8 hours/day, 10 days/week from conception to postnatal day 18. Mothers and litters, which were paired to 8 pups, were exposed on the same schedule until day 25 when the pups were weaned. The pups alone were exposed until day 30. Male offspring were tested on day 55 for locomotor exploration of a novel T-maze. The halothane-exposed rats (N=12 litters) showed significantly slower speeds than control rats (N=12 litters), in locomotors from the start box at the bottom of the stem into either of the arms. These data indicate that the effects of halothane are desensitizable with exposure to only 30 days after birth and that the behavioral effects are not confined to high motivation tasks. Low motivation tasks like locomotor exploration also appear to be sensitive indicators of behavioral toxicity.
MECHANISMS OF AFFERENT LAMINATION IN DEVELOPING HIPPOCAMPUS REVEALED BY OUTGROWTH OF FIBERS FROM SEPTAL IMPLANTS. E. R. Lewis* and E. W. Conner** (P. T. Kelly), Dept. of Psychobiology, U.C. Irvine, CA 92717

Pieces of embryonic septal tissue were implanted into the entorhinal or occipital cortices of neonatal rat hosts (2–3 days old). The initial outgrowth of fibers originating from the graft and their pattern of termination within the host hippocampal formation was examined using histochemical staining for acetylcholinesterase (AChE). Firnbrai transplants performed in conjunction with the implant surgery eliminated native septohippocampal fibers. When placed in the entorhinal cortex, septal implants preferentially invaded the normal deep laminar zones of the host hippocampal and dentate gyri. None of the laminar zones that receive septal efferents in the normal animal were invaded by septal implants placed in the entorhinal cortex. A cholinesterase staining pattern similar to that observed in the normal hippocampus was evident in the dentate gyrus of implanted animals, both in the unimplanted control hemisphere and in the hemisphere ipsilateral to the implant. The only septal innervated laminae were those already innervated by septal efferents. The major premise of this study is that the position of afferent source and the time of arrival of fibers from the graft control the organization of the hippocampal and dentate laminae.

A GOLGI STUDY OF DEVELOPING CHICK SYMPATHETIC NEURONS. L. Luckenhoff, M. J. B. Fleury, and J. W. McCann. Dept. Zoology & Microbiology and one animal displayed AChE reaction product in the dentate molecular layer. Processes emanating from cells in the implant were oriented in the direction of the hippocampus throughout the developmental period. In one group of animals, the fibria was left intact for 28 days after the implant surgery in order to examine whether interconnections between fibers from the implant and native septohippocampal efferents. Extremely light AChE staining is present in the hippocampus and dentate gyrus of animals in this group sacrificed at E7, 14, and 30 days postimplantation. Fibers were severed. When tissue from the striatal region of the embryonic brain was implanted into the entorhinal cortex, AChE reaction product in the hippocampus is restricted to the outer third of the dentate molecular layer.

The position of the afferent source and the time of arrival of the different fibers from the graft seem to be critical to the mechanisms underlying the development of specific connection patterns in this system. Temporal competition between homologous afferents and a hierarchical scheme of growth possibilities appear to play a role in developmental specificity. In addition, we have proposed that contact between a "critical afferent" (the CA system in this case) and the target cell might "set" the dendritic membrane for the appropriate patterns of connectivity. (Grants NS 08597 and MH 16691).

547 EFFECTS OF EXPOSURE TO HORIZONTAL OR VERTICAL STRIPES DURING REARING ON EvOKED POTENTIALS TO THESE STIMULI AT MATURITY IN RATS. Jill London*, Howard Rowley, Gregory McCarthy, and William T. Greenough (SPON: E. Donchin). Dept. of Physiol. and Psychol. and Neural & Behav. Biol. Prog., Univ. of Ill., Urbana, IL 61801.

Studies in cats (e.g., Blakemore & Cooper, Nature, 1970,  273: 373) indicate that orientation specificity of receptive fields of visual cortex neurons can be altered by rearing in artificial visual environments. We have investigated the effects of early exposure to a striped visual field if the cats are exposed exclusively to a single orientation during development. We have examined this phenomenon in rats. Two experimental procedures were utilized to expose potential differences to the exposed and orthogonal orientations.

Breded rats of both sexes were reared from birth in the dark. Beginning at the day of birth, one group was exposed to square 13 d) and continuing to day 52, 724 rats were reared for 1 hr. per day 5 d. per week to a field of alternating black and white stripes of (variable thickness) oriented either vertically or horizontally. In the former condition, the animals were exposed to a striped field up to the day of sacrifice. When exposed to the white squares of the black-white pattern, the animals were wrapped in gauze and placed in boxes with their heads exposed. The boxes were secured to the side of the cage by a cloth strip. Except for these exposure periods, animals were kept in darkness (or dim red light during brief daily maintenance) throughout the experiment.

The stainless steel screw electrodes were implanted unilaterally or bilaterally over areas 17-18 of the cortex on day 52. After 2-7 days, unanesthetized animals were secured to a board facing either a vertical or horizontal stimulus field in the dark. Evoked potentials to strobe illumination of the field at 1 sec. intervals were averaged with a Fabrich signal averager and additionally in some cases with a computer averaging program.

NI-P2 peak to peak amplitude was measured from visual evoked potentials to 64 presentations of vertical and horizontal fields from each side of the head of exposed animals. The data from 7 of 8 comparisons indicated greater amplitude to the horizontal stimulus with a mean difference of 172 (p<.01). For vertically exposed animals, fields of view were vertically oriented. Preliminary work indicates mean amplitude of evoked potential amplitude to the two orientations in dark and cyclic light reared animals. Studies of evoked potentials to near square wave gratings are in progress.

Supported by MS 79-182525.

The sensory neurons in the antenna of the moth Manduca sexta mature and send their axons into the CNS early in the 18 days of adult development. Intronervation in the antennal lobes (AL) of the brain also develop at this time. This study examines the morphological and electrophysiological development of the AL interneurons and their interactions with synapses.

Using intracellular recording and dye-injection techniques, we have classified neurons in the AL according to their morphological and physiological properties. Cells in mature moths were tested for their responses to antennal stimulation with female sex pheromone, general odorant (z-myo-2-hexenial), and mechanical stimuli. At least 2 broad categories of interneurons can be discerned morphologically: "local" interneurons with multiglomerular arborizations and no axons, and "output" interneurons with arborizations in single glomeruli and axons projecting out of the AL to the corpora pedunculata. Both of these classes can be subdivided in males with respect to responses to pheromone; no female neurons respond to pheromone. Pheromone-responsive interneurons and output interneurons in a macrogglomerular complex found only in the male AL.

Intracellular recordings from AL neurons in developing moths first detect synaptic transmission about day 9 (midway through adult development). The incidence and strength of synaptic transmission increase sharply over the following 4 days, coinciding with the final development of increased levels of serotonin and acetylcholine in the AL (Brain Res. 179 389, 1977) and with the appearance of mature synaptic profiles in electron microscope preparations of the olfactory neuropil (Tolbert, 1977, Phil. Trans. Roy. Soc., volume). By day 13 the development of synaptic transmission, as monitored by intrasomatally recorded electrical stimulation of afferents, is essentially complete. The appearance of the morphological arborization of the interneurons appears to coincide with their electrophysiological maturation. The multiglomerular local interneurons develop by progressive elaboration of their arbors and the appearance of additional-like structures. The output interneurons initially develop multiglomerular arbors that subsequently retract between days 12 and 14 to leave a single-glomerular dendritic tuft that continues to increase in density.

(Supported by NSF Grant BNS77-13281 to J.G. Hildebrand and an NIH postdoctoral fellowship to G.M.)

551 EFFECT OF RE-INNERRVATION, DEVELOPMENT AND DEGENERATION ON MEPP AMPLITUDE DISTRIBUTIONS IN THE MOUSE DIAPHRAGM. C.G. Muir* and C.G. Carlson* (SPON: N. West) Dept. Physiol., Upstate Medical Center, Syracuse, NY 13210

It has been established that there are two classes of MEPPs in the mouse (Kriebel, Lladós & Matteson, 1976, J. Neurophysiol.). Two classes of MEPPs have also been seen in the re-innervating mouse diaphragm. (Dennis & Miledi, 1974, J. Physiol.).

We have found in the re-innervating mouse diaphragm that the number of small mode MEPPs relative to major mode MEPPs is often greatly accentuated. Some cells which are newly re-innervated show a clear normal distribution of small mode MEPPs and a few with a mean 10-12 times the size of the small mode MEPPs. As the process of re-innervation progresses the percentage of major mode MEPPs increases with respect to the small MEPPs. A gradient is often seen in a single preparation in which the fibers near the point of nerve entry into the muscle have many major mode MEPPs and relatively few small mode MEPPs, while penetrations far from nerve entry give predominately small mode MEPPs.

In preparations partially blocked with cobalt it was found that the unitary evoked response was always greater than the major mode of the MEPPs.

After cutting or crushing the phrenic nerve spontaneous activity ceased within 18 hours. Changes in MEPP distributions in these preparations are rare.

Some MEPP distributions of the newborn mouse diaphragm, show a similarity to the re-innervating preparations described above.

These results indicate that there are two types of release processes at the neuromuscular junction that develop at different rates. (Supported by NIH Grant 11-15240)

552 DEVELOPMENT OF DOPAMINE AND NEUROLEPTIC RECEPTORS IN THE CNS: IN VIVO STUDIES. J. Charles Murray, Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE, 68103.

A number of laboratories have demonstrated the feasibility of studying dopamine (DA) and neuroleptic receptors in vivo in adult rat brain by the use of radioiodinated DA antagonists, such as 3H-spiperone (3H-Sp) (e.g. Life Sciences 22: 203, 1976). These studies have substantiated regional distributions of stereospecifically bound 3H-Sp that was consistent with in vitro studies and with known DA innervation of brain regions. The in vivo binding was saturable and was blocked by DA agonists and antagonists. We here report the adaptation of these in vivo binding techniques to the study of dopamine and neuroleptic receptors in vivo in the developing neonatal rat brain.

Rats pups 5 days old were injected intraperitoneally with 3H-Sp (1 mg/kg, 10% saline) and then sacrificed at various times post injection, typically at 1, 2, 4 and 6 hours. Brain tissue was assayed by liquid scintillation counting for amounts of 3H-Sp bound. In 5 day old rat pups there was a regional distribution of 3H-Sp binding. The highest level of binding was observed in the striatum again demonstrating the greatest level of binding. By day 15 specific binding could also be demonstrated in olfactory bulbs, cortex, hippocampus, hypothalamus, cerebellum, thalamus and brain stem. Striatal/cerebellar ratio was 8:1. In 15 day rats similar results were obtained with (-)-butaclamol (5 mg/kg, i.p., 30 min. before 3H-Sp). (-)-Butaclamol had no effect on levels of 3H-Sp bound in any region. In the striatum binding reached maximum levels at 1 hour and remained at this level for 4 hours. At 4 hours the striatal/cerebellar ratio was 8:1. In 15 day rats similar results were obtained with striatum again demonstrating the greatest level of binding. By day 15 specific binding could also be demonstrated in olfactory bulbs, cortex, hypothalamus, thalamus and brain stem. Striatal binding reached its maximum at 1 hour and remained at these levels for 2 hours, at which time the striatal/cerebellar ratio was 8:1. Binding in all brain regions at 15 days was reduced to control levels by (-)-butaclamol and unaffected by (-)-butaclamol. These studies demonstrate the usefulness of i.p. injection for in vivo studies of dopamine and neuroleptic receptors in neonatal rats. They are consistent with the general posterior to anterior pattern of dopaminergic development as demonstrated by fluorescence histochemistry. They are also in good agreement with in vitro studies of the ontogeny of DA receptors in the striatum (Brain Res. 125: 376, 1977) showing a large increase in the level of DA receptors for binding between day 5 and day 15. The applicability of these data to autoradiographic studies will be discussed.

Supported by a seed grant from the Univ. of Nebraska.


In a previous study, we described three distinct periods in the development of spontaneous oral area activity in rat fetuses: (1) an early phase, beginning from day 17; (2) an acceleration phase, reaching a peak of activity on day 18 through day 19; and (3) a deceleration phase, from late day 19 through day 20. Components of this acceleration phase were based on the development of spontaneous oral area activity in rat fetuses. Different methods were used for different activities. The activity of the mouth and forelimb during development is less clear. In the present study, we have investigated the sequential appearance of coordinated activity between mouth and forelimb during development. An aspiration rate was used for the analysis of interrelations and control of neural networks involved in this early appearing pattern of behavior. Fetuses ranging from 14 to 20 days gestation age were used in this study. Spontaneous and evoked activity were recorded both quantitatively and qualitatively.

Forelimb movements first appeared as part of a total pattern with head movements until day 19. This was followed by local forelimb movements from day 17 of gestation age. A distinct synchronization of forelimb and mouth activity was observed in fetuses of 18 days gestation age. These movements consisted of alternation of flexion and extension of the forelimbs, and face wiping. The forelimb movements were accompanied by mouth opening. Each time the forelimb was extended in the direction of the head, after day 18, fine digit movements were noticeable with an increasing frequency of synchronized movements of forelimbs, mouth opening and head movements. The movement of the head is greatly accentuated. Some cells which are newly re-innervated show a clear normal distribution of small mode MEPPs and a few with a mean 10-12 times the size of the small mode MEPPs. As the process of re-innervation progresses the percentage of major mode MEPPs increases with respect to the small MEPPs. A gradient is often seen in a single preparation in which the fibers near the point of nerve entry into the muscle have many major mode MEPPs and relatively few small mode MEPPs, while penetrations far from nerve entry give predominately small mode MEPPs.

In preparations partially blocked with cobalt it was found that the unitary evoked response was always greater than the major mode of the MEPPs. After cutting or crushing the phrenic nerve spontaneous activity ceased within 18 hours. Changes in MEPP distributions in these preparations are rare. Some MEPP distributions of the newborn mouse diaphragm, show a similarity to the re-innervating preparations described above. These results indicate that there are two types of release processes at the neuromuscular junction that develop at different rates. (Supported by NIH Grant 11-15240)

Conjoint twins occur occasionally during development by avian species due to duplication of the primitive streak. In a batch of duck embryos raised from incubating eggs, we found a duck embryo of 16 days incubation age with two bills as the only outward sign of duplication. The two bills were of equal size, each with fully developed upper and lower beaks. No beak movements were observed as in normal embryos at corresponding stages of development. The abnormal embryo had two eyes which were normal in outward appearance and were symmetrically placed one on either side of the head. Dissection of the head revealed, however, duplication of the telencephalon, optic lobes and the rhombencephalon. The inner (medial) half of each of the duplicated embryonic brain division was greatly reduced. Histologic examination of sections through the head revealed the presence of two additional optic primordia each consisting of an undifferentiated mass of tissue representing lens and a much folded epithelium. The orbital muscles except for those associated with the pair of normal eyes were greatly increased. Abnormal differentiation of cleft centers such as the ciliary ganglia, trochlear and accessory oculomotor nuclei and mesencephalic nucleus of the trigeminal nerve was determined by the number of the four ciliary ganglia, four and 200R Co60 irradiation effects on growth, behavior and brain cells in the mesencephalic nucleus of the abnormal embryo. The nucleus was slightly less than normal in spite of the duplication. The primary duplication which could account for the absence of the mesencephalic nucleus was slightly more than half normal in spite of the duplication affecting the bill. The masseter muscles did not show a corresponding duplication which could account for the absence of beam movements and for the slight decrease in the number of cells in the mesencephalic nucleus of the abnormal embryo.

Supported by the National Institutes of Health—National Institutes of Child Health and Human Development #R01-HD12064.

Interaction effects of prenatal co60 irradiation and postnatal nursing or mother rearing on growth, behavior, and persistence of impairment in squirrel monkey offspring. J. M. Ordy, K. R. Brizée, J. Beavers and P. Medart† Tulane University, Delta Regional Primate Research Center, Covington, La. 70433.

For a variety of phylogenetic and logistical reasons, the squirrel monkey has rapidly become one of the more widely used primate species for studying the effects of environmental hazards from birth. The aim of this study was to identify and segregate the factors in the use of this diurnal primate model include: (1) the species of the operates and, as such, were perhaps better able to compete for food. In contrast the binaural controls were comprised by a series of 3 different litters, while another entire litter comprised the experimental group. Therefore, all the binaurally-deprived pups had relatively better nutritional opportunities than the binaurally-operated subjects. In any case, sham operates should be included in future experiments of this type.


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Electron micrographs of 200 µm2 systematic samples of the dorsal cornuate nucleus near the obex, from pouch young opossums, in the period prior to and during initial synaptogenesis at 8 and 9 days after birth, were analyzed planimetrically. At 3 days postnatal, during the period of epithelial cell division, perikaryal cytoplasm (F) and nuclei (H) accounted for 93% of the identified area in the sample; profiles containing a floccular appearing substance and an electron-lucent background (ELF), possibly dendritic growth cones, were 22%; profiles of processes containing microtubules and ribosomes (MTR), possibly neurites, made up 4%; and profiles containing microfilaments (MF) made up the remaining 2%. At 5 days, when cell migration is under way, P and N made up 39% and 35% respectively, axonal growth cones and/or degenerating processes were 64%, ELF 18%, MTR 3%, MT 3%, AT 0% less than 1%, ED 6%, and MF 22%. Axonal endings with round vesicles (AT) appeared on the 6th day, and large profiles containing a floccular appearing matrix and sparse or clumped synaptically-sized vesicles (ATL) were identified in the 8 and 9 day samples. At 8 days P were 23%, N 19%, ELF 23%, MTR 8%, MT 5%, AT 3%, ATL 28, ED 4%, MF 15%. At 9 days P were 26%, N 18%, ELF 14%, MTR 21%, MT 17%, AT 4%, ATL 4%, ED 4%, and MF 0%. These findings are consistent with a sequence of events wherein dendritic growth has started on the third day and peaks on the 8th day; axonal growth cones appear on the 5th day and peak on the 7th day; both of these peaks are just prior to synaptogenesis on the 8th and 9th days. Postsymptomatic profiles then include MTR, N, ELF, and P; presymptomatic profiles are ED, AT and ATL. (Supported by NIH Grant NS 05982.)


Small extracellular electric fields have been found to influence the neurite growth from chick medulla and dorsal root ganglia explants (Marsh and Beams, J. Cell. Comp. Physiol. 27: 139, 1946; Jaffe and Poo, J. Exp. Zool., 1979, in press). In the present study, we found that an extracellular electric field of about 60 µm greatly enhanced the axonal growth of dissociated spinal cord neurones by the retino-cerebellar-type culture. Single nerve cells of 1-day old Xenopus embryos (Stage 17-19) were cultured on a clean, glass surface by previously described method (Patyi and Poock, Nature 230, 1971). After the cells adhered to the glass substratum, a uniform, steady electric field was applied to the culture via a pair of agar bridges filled with a phosphate saline. The position of the growth cones with respect to the soma was recorded in polar coordinates after the field was removed. In the absence of an electric field (control), there was a radially symmetrical growth of axons with an average growth rate of 11.7 ± 0.9 µm/hr (± S.E.M., N = 53). When an electric field was applied for 6 hrs, in 3 separate experiments, there was a marked asymmetric growth. The average axonal growth rate towards the cathode was 20.6 ± 1.5 µm/hr (N = 85); while towards the anode it was 12.6 ± 1.1 µm/hr (N = 53). It was observed that after the onset of the field, the growth cones of axons previously extending perpendicularly to the field axis gradually changed their direction of growth towards the cathode. In addition, neurones with no previous axonal growth now grew their neurites predominantly from the cathodal pole of the soma. This indicates that the electric field produces its orienting effects both in the rate and direction of axonal growth, and in the initial point of origin of the axon from the soma. Since the electric field used was too small to act directly on the intracellular components, we hypothesize that its effects are mediated through the lateral electrophoresis of membrane-bound growth-controlling components along the plasma membrane.

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The effects of rapid and specific depletion of plasma levels of tyrosine (Tyr) and phenylalanine (Phe) between 4 and 8 days of age on subsequent behavior were studied in rats. Phenylalanine ammonia-lyase (PAL) is a powerful enzyme which catalyzes the degradation of Phe to tyrosine. Rats were treated with PAL at 4 or 8 days of age. Treatment significantly reduced brain levels of Tyr and Phe which averaged 89% of controls. PAL rapidly and selectively depletes plasma of these amino acid precursors of catecholamines (CA) synthesis (Abell, et al., 1973; Barratt, et al., 1976). As a result brain levels of CA’s are reduced (Barratt, et al., 1976).

Daily injections of 100 units PAL per kilogram body weight (Experimental) and buffer vehicle (Control) were administered I.P. to neonatal rats between 4 and 8 days of age (Inclusive). The animals were weighed at 22 days of age and housed multiply thereafter. Locomotor activity testing at 39 days of age revealed that PAL-treated animals exhibited a decrease in the time spent active, as measured initially (first five minute period) but were no different than controls during two subsequent five minute periods. This difference was statistically significant. PAL rats were also defeated less in the test apparatus than controls, a finding which also was statistically reliable. Differences in shuttle avoidance acquisition were not statistically reliable. PAL rats demonstrated a greater number of total inter-trial shuttle crossings than controls. No weight differences were seen at time of testing between the groups.


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IMMUNOCYTOCHEMICAL LOCALIZATION OF ENKEPHALINE AND SUBSTANCE P

Using immunocytochemistry, we sought to determine the presence and regional distribution of neurons containing enkephalin and substance P (SP) at embryonic (E) days 15, 18 and 20, and postnatal (PN) days 1 and 21. Adjacent sections for both peptides were incubated with specific antisera to enkephalin, SP or control serum and then processed by the peroxidase-antiperoxidase method. The reaction product for both peptides was absent at E15 and was present in certain groups of perikary and fibers at E18. At the latter age, enkephalin-like immunoreactivity (ELI) was located to perikarya in many regions of the chick brain. At PN1, ELI was not confined to SP labeled neurons. These include substantia gelatinosa of n. trigeminal, n. tractus solitarius, n. lateral lemniscus, and n. parabrachialis. Other regions such as n. stria terminalis, caudate-putamen, and n. central amygdala contained perikarya for both SP and enkephalin; whereas the n. habenularis lateralis contained only SP labeled perikarya. Fibers, but not varicose terminal fields, showed enkephalin and SP reaction product at the E18 stage. Terminal regions showing both enkephalin and SP were present by E20. The location of terminals and perikarya containing ELI at PN was similar to that of adult colchicine treated rat brains (Sar et al., J. Comp. Neurol. 182: 17, 1978). Although the nuclear groups contained more perikarya selectively labeled for the peptides at PNl, their distribution was essentially the same as E20. Both ELI and SP were present in well defined fiber bundles. ELI was more abundant in numerous locations, some of which were also common to SP including the lateral lemniscus, medialis forebrain, and stria terminalis. Other bundles such as the mamillothalamic contained only ELI, while certain fiber systems of the habenula or peruncinal contained only SP. There were likewise similarities and differences in the terminal fields labeled with SP and/or enkephalin at PNl. We conclude that 81- or 82-SP receptors results similar to that observed for total 8-adrenergic receptors, the density of cerebellar 81-SP receptors was significantly decreased by the day after hatching. The development of the density of the two receptor subtypes closely paralleled the development of total 8-receptors in the cerebral cortex.

The ontogeny of 8-adrenergic receptors in the cerebral cortex, which contains mainly 82-receptors, was strikingly different from that observed in the cerebral cortex. Total cerebellar 8-adrenergic receptor density steadily increased from postnatal day 3 through day 42. At this time the density of receptors plateaued and remained constant for up to six months. The relative proportions of 81- and 82-receptors in the cerebellum changed markedly during development. Between days 8 and 13 approximately 18% of the receptors were of the 81 subtype. This proportion steadily decreased with age, and in 3 to 6 month old animals only 2% of the receptors were of the 81 subtype. Although the density of cerebellar 81-adrenergic receptors showed similar to that observed for total 8-adrenergic receptors, the density of cerebellar 82-adrenergic receptors was steadily declined between days 20 and 90. The relationship between the development of 81- and 82-receptors and various developmental events in the cerebral cortex and cerebellum will be discussed.

(Supported by N I H Grants NS 24285, NS 09611, NH 18974, NH 00078, and NS 00121. SP antiserum generously supplied by S. E. Leeman, New York, N.Y. 10021.)


The 8-adrenergic receptor antagonist 1,25-dihydroxydexamethasponindolindol (IYP) has the same affinity for 81- and 82-adrenergic receptors. Graphical analysis of the inhibition of specific IYP binding by a variety of compounds selective for 81 or 82-receptors, was used to determine the densities of the two receptor subtypes in homogenates of rat cerebral cortex and cerebellum at various time points during development. In the cerebral cortex, which contains mainly 81-receptors, total 8-adrenergic receptor density increased sharply between postnatal days 10 and 21. The density of receptors remaining fairly constant through six weeks of age though it subsequently declined. The relative proportions of 81- and 82-receptors were fairly constant throughout development of the cerebral cortex. Therefore, the development of the density of the two receptor subtypes closely paralleled the development of total 8-receptors in the cerebral cortex.


The chick spinal cord receives a substantial descending adrenergic input from supraspinal levels. It has been recently reported that during development these descending adrenergic afferents contain noradrenergic and 81-adrenergic fibers. We studied the development of these synapses, we studied the development of adrenergic synapses in the chick spinal cord. It appears that the early development of adrenergic synapses might not be dependent on the presence of 8-adrenergic receptors on the spinal cord neurons. The role of the adrenergic input in the development and maintenance of 8-adrenergic receptors is under investigation.

Supported by: NIH Grants NS 13768 and NS 07061.

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THE ELIMINATION OF REDUNDANT INNERVATION IN NEONATAL SYMPATHETIC GANGLIA. Dale Purves and Jeff W. Lichtman. Dept. of Physiol. and Biophy., Washington Univ. Sch. of Med., St. Louis, MO 63110

Neurons in superior cervical ganglia from neonatal (0-7 day) hamsters are innervated by an average of 11-12 axons arising from 4 different spinal cord segments. Ganglion cells from adult animals, however, are innervated by only 6-7 axons arising from about 3 spinal cord segments. Thus many of the synaptic contacts initially present appear to be lost during development. This elimination of redundant innervation probably occurs during the first few weeks of postnatal life since ganglion cells in 2-3 week-old animals are innervated to an intermediate degree. The overall innervation of the superior cervical ganglion in adult hamsters arises from thoracic segments T1-T5; in spite of the loss of about half of the innervating axon terminals, no additional spinal segments contribute significantly to the innervation of ganglia in neonatal animals. Thus synapse elimination does not occur because some initial innervation arises from inappropriate spinal levels. As in other mammals (see for example Rij and Purves, 1977) the pattern of adult ganglion cell innervation in the hamster is selected and tends to receive innervation from a preferential subset of the segments that innervate the ganglia as a whole. This selective tendency is less pronounced in the neonatal animal, and we conclude that a) the elimination of redundant innervation in early life is not limited to target cells innervated by a single axon in maturity, and b) the loss of redundant contacts refines the selective pattern of synapses made on individual ganglion cells.


BEHAVIORAL COMPONENTS OF THE CLONIDINE RESPONSE IN THE DEVELOPING ADRENERGIC SYSTEM. may depend on the maturational state of the organization and coordination. The behavioral effects of this adrenergic system may depend on the maturational state of the corticospinal tract.

571 THE EFFECT OF CORDOTOMY ON DENDRITE BUNDLES AND TREADMILL WALKING IN KITTENS. Patricia Rebach* (Sponsor: B.D. Lindsay). Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

Dendritic bundles of a motoneuron are present in the spinal segments innervating the forelimbs, but not the hindlimbs of the cat at birth. Since bundles mature in the lumbar sacral area coeval with the ability to walk, Scheibeil and Scheibel (Exp. Neurol. 21:106 1970) have suggested that dendritic bundles might be a substratum for spinal activity that helped to shape motor control, especially in the coordination of locomotion.

The present study addresses the problem of dendrite bundle formation and maintenance in relationship to treadmill walking of cats, with spinal cord transections at T-12 made during 2 stages of development: 2 weeks postnatal, at which time dendritic bundles and alternating flexor/extensor movements are rudimentary and 12 weeks postnatal, a time when dendritic bundles and walking ability approach that of the mature cat. Each age group was separated into 2 subgroups: those exercised daily for 20 minutes of treadmill walking and those left to spontaneous activity in a limited environment. Kittens were sacrificed 6 weeks or more post-cordotomy. Prior to sacrifice, both nonexercised and exercised groups were rated on a scale of 1-7 for ability to walk on a motorized treadmill at several speeds. At sacrifice the animals were perfused and the degree of bundle formation observed by light microscopy. Spinal segments on coded slides were rated on a scale of 0-5 for presence and complexity of bundles. This data was then compared to the treadmill rating.

Preliminary results suggest that CNS integrity is not always a prerequisite for bundle formation and that dendrite bundle formation and/or maintenance in the lumbar cord are not necessary requirements for treadmill walking. Although there was evidence of bundle formation in 6 out of 8 of the animals cordotomized at 2 weeks (ratings ranged from 1-4), this formation was not correlated to ability to treadmill walk or to spontaneous weight bearing. There was also no correlation between bundle formation and treadmill exercised animals. Cordotomy on the 12 week animal had a detrimental effect on maintenance of the bundles. These animals were all sacrificed after 6 months of age, at which time, ordinarily, bundle formation is within the 4-5 classification (mature). None of the sections examined were rated higher than a 3 on the numerical scale, and signs of degeneration were readily apparent in all sections. Again, no correlation between the treadmill rating and degree of bundling was observed. Initial evidence, however, suggested that daily exercise on the treadmill may slow degeneration of the bundles. Supported by a grant from Easter Seal Research Foundation R7112.

572 DEVELOPMENTAL PATTERN AND LOCALIZATION OF A RAT BRAIN SPECIFIC ANTIGEN (G5) USING MONOCLONAL ANTIBODY. Jane Somel Rodman* and Richard Akeson, Children's Hospital Research Fdn., Cincinnati, Ohio 45229.

G5 is a monoclonal antibody developed from the fusion of a myeloma cell line (P3-X63-Ag8) and spleen cells from a mouse immunized with rat pheochromocytoma cell line (PC12). G5 reacts to a protein that is specifically synthesized by PC12 cells and is present in lesser extent with rat spleen and bone marrow but not with other tissues or cells as has been previously described (Akeson and Graham, submitted). An increase specific binding activity was observed in extract of 300-400 mg of brain particulate protein from 30-40-day-old rats, and this binding was increased in the newborn rat brain (30 mg IgG bound/mg brain particulate protein). G5 binding increases rapidly with age until day 25 to levels of 300-400 ng IgG bound/mg brain particulate protein. After this time, G5 specific binding activity increases slowly to adult levels of 400-500 ng IgG bound/mg brain particulate protein.

The developmental pattern of G5 antigen has been studied using the direct binding assay on homogenates of regions of the dissected adult rat brain. It has been found that G5 antigen is very low in the newborn rat brain (30 mg IgG bound/mg brain particulate protein). G5 binding increases rapidly with age up to day 25 to levels of 300-400 ng IgG bound/mg brain particulate protein. After this time, G5 specific binding activity increases slowly to adult levels of 400-500 ng IgG bound/mg brain particulate protein. The total G5 binding to brain increases 150-200 fold from birth to age 60 days.

The macroscopic localization of G5 antigen has been studied using the direct binding assay on homogenates of regions of the dissected adult rat brain. It has been found that G5 antigen is concentrated in the cerebral cortex gray matter which contains ten-fold more G5 antigen than cerebellum, cerebral cortex white matter, brain stem or spinal cord.

Additional studies to determine the cell type specificity and molecular composition of G5 antigen will be reported.

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In the developing mouse gut neurons cannot be detected morphologically by light or electron microscopy prior to day 12 of gestation. Before this time the neuronal mesenchymal tube surrounding a stratified mucosal epithelium and encompassed by a subjacent layer of eosinophilic lateral cells. The gut shows no neuronal properties. It fails to synthesize H-acetycholine (H-ac) from H-choline; it fails to specifically take up H-5-hydroxytryptamine (H-5-HT), and neither H-5-HT nor norepinephrine (NE) can be shown to influence histofluorescence. Neurons first appear morphologically in the small intestine at 12 days' gestation, at 13 days in the stomach and proximal colon, and at 14 days in the distal colon. Coincidentally, with the appearance of neurons, synthesis of H-ac from H-choline appears and uptake of H-5-HT becomes radioautographically demonstrable in the primordial myenteric plexus. When any region of the gut is explanted in organotypic tissue culture prior to neuron formation, neurons subsequently develop. This can be shown as early as 10 days' gestation but also occurs at later ages. In the cultures the epithelium degenerates but two layers of smooth muscle cells invade and colonize the gut substantially before neurons can be recognized chemically or morphologically. This indicates that differentiation of enteric neurons occurs within, and is probably dependent upon, the enteric microenvironment. (Supported by a Basil O'Connor Starter Research Grant, National Foundation - March of Dimes and NIH Grant NS12969.)


We have previously described by morphometric analysis (Ross et al., 1978) that few synapses are found in the chick lumbar sympathetic ganglia prior to hatching. Beginning at two days post-hatching, there is a rapid proliferation of synapses and adult levels are attained by 21 days. We have also reported (Ross, Smolen and Cosio, 1977) that spinal cord transection in the chick embryo at the presynaptic stage (2 days in ovo), while reducing the afferent input to the preganglionic nucleus, does not affect either the number or the morphological development of the neurons of this nucleus.

To determine the effects of supraspinal input to the preganglionic neurons on the development of peripheral ganglionic synapse formation, the spinal cords of two days old chicks were transected at cord level T3 and the animals were allowed to survive until 21 days. At this time the chicks were perfusion fixed and the lower thoracic spinal cords and the lumbar ganglia were prepared for ultrastructural morphometric study.

In the spinal cord transected chicks, the synaptic density in the ganglia was reduced by 60% when compared with controls of the same age. Although the weight of the spinal cord transected animals were considerably smaller than those of the controls, the density of neurons was not significantly different. These observations provide a morphometric extension of the biophysical observations of Hamill, Bloom and Black (1977) and confirm the existence of a transsynaptic influence on the formation of ganglionic synapses. Bregma, Lambda corresponded to the caudal tip of the occipital lobe, and to the frontal bone of the brain, respectively. The major modification of the Kopf piglet stereotaxic apparatus (Goottman et al., Am. J. Physiol., 221: 994, 1972) was the use of straight ear bars directly into the ear canal. These ear bars probably fill the gap in the ventral half of the cribiform plate and permit the head to be held in a consistently reproducible position. The position of the brain was standardized by keeping Lambda and Bregma at the same vertical level from stereotaxic zero by lowering or raising the palatal-mass piece, in this way the surface of the brain remained parallel to the horizontal zero of the stereotaxic instrument.

Growth of the skull and brain was measured as distances between Lambda, Bregma and Nasal sutures to the stereotaxic zero. Lambda corresponded to the caudal tip of the occipital lobe. Bregma to the level of the medial thalamus and the Nasal suture to the anterior tip of the frontal lobe.

Four major groups were found to have significant differences in brain size:

<table>
<thead>
<tr>
<th>Ages (days)</th>
<th>Weight (kg)</th>
<th>Lambda (mm)</th>
<th>Bregma (mm)</th>
<th>Nasal (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1</td>
<td>1.4 ± 0.09</td>
<td>10.950 ± 0.62</td>
<td>48 ± 2.2087</td>
<td>43.7 ± 0.12</td>
</tr>
<tr>
<td>2-5</td>
<td>1.190 ± 0.07</td>
<td>10.770 ± 0.17</td>
<td>41 ± 0.4713</td>
<td>44.3571 ± 0.29</td>
</tr>
<tr>
<td>6-9</td>
<td>1.8 ± 0.18</td>
<td>10.120 ± 0.93</td>
<td>43 ± 0.4713</td>
<td>49.5126 ± 0.41</td>
</tr>
<tr>
<td>10-12</td>
<td>2.950 ± 0.31</td>
<td>14.7 ± 0.682 ± 0.35</td>
<td>49.7 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

The map containing these values will facilitate stereotaxic localization of brain structures in piglets of different ages. (Supported by NIH Research Grant HL-20864.)


To determine the effects of supraspinal input to the preganglionic neurons on the development of peripheral ganglionic synapse formation, the spinal cords of two days old chicks were transected at cord level T3 and the animals were allowed to survive until 21 days. At this time the chicks were perfusion fixed and the lower thoracic spinal cords and the lumbar ganglia were prepared for ultrastructural morphometric study.

We have demonstrated that retinal ganglion cell axons are distributed non-randomly in the optic nerve of goldfish, and that one of the rules for their ordering is: axons from ganglion cells of the same age, that is, located the same distance from the optic disc, remain together. The non-random distribution of axons in the optic nerve was demonstrated with small injections of HRP into the retina. Injections were made peripherally so that axons from a localized region rather than axons of passage, were filled. Fish survived 2-8 days, depending on their size; retinas were prepared as whole mounts, and optic nerves and tecta were sectioned longitudinally; the tissue was reacted with H2O2 and EY00168 to S.S.E.)

The major modification of the Kopf piglet stereotaxic apparatus (Goottman et al., Am. J. Physiol., 221: 994, 1972) was the use of straight ear bars directly into the ear canal. These ear bars probably fill the gap in the ventral half of the cribiform plate and permit the head to be held in a consistently reproducible position. The position of the brain was standardized by keeping Lambda and Bregma at the same vertical level from stereotaxic zero by lowering or raising the palatal-mass piece, in this way the surface of the brain remained parallel to the horizontal zero of the stereotaxic instrument.

Growth of the skull and brain was measured as distances between Lambda, Bregma and Nasal sutures to the stereotaxic zero. Lambda corresponded to the caudal tip of the occipital lobe. Bregma to the level of the medial thalamus and the Nasal suture to the anterior tip of the frontal lobe.

Four major groups were found to have significant differences in brain size:

<table>
<thead>
<tr>
<th>Ages (days)</th>
<th>Weight (kg)</th>
<th>Lambda (mm)</th>
<th>Bregma (mm)</th>
<th>Nasal (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1</td>
<td>1.4 ± 0.09</td>
<td>10.950 ± 0.62</td>
<td>48 ± 2.2087</td>
<td>43.7 ± 0.12</td>
</tr>
<tr>
<td>2-5</td>
<td>1.190 ± 0.07</td>
<td>10.770 ± 0.17</td>
<td>41 ± 0.4713</td>
<td>44.3571 ± 0.29</td>
</tr>
<tr>
<td>6-9</td>
<td>1.8 ± 0.18</td>
<td>10.120 ± 0.93</td>
<td>43 ± 0.4713</td>
<td>49.5126 ± 0.41</td>
</tr>
<tr>
<td>10-12</td>
<td>2.950 ± 0.31</td>
<td>14.7 ± 0.682 ± 0.35</td>
<td>49.7 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

The map containing these values will facilitate stereotaxic localization of brain structures in piglets of different ages. (Supported by NIH Research Grant HL-20864.)

576 DEVELOPMENT differentiación de neuronas entéricas de procedencia no reconocida. Leonard L. Ross et al. 1978), ha hecho necesario el desarrollo de un atlas correlativo, que relacione el crecimiento del cráneo con las estructuras de la evolución del cerebro. Para este estudio se utilizaron 33 cerdos de 3hr-12 días, de peso medio 0.74-3.65 kg, que se mantuvieron en una celda de cultivo, preparando muestras de tejido cerebral. Las uniones sinápticas que se formaron fueron radioautografiadas para determinar la edad de los axones. Se observó que las uniones sinápticas inferiores se formaron a partir de los 12 días de vida, mientras que las uniones sinápticas superiores se formaron a partir de los 21 días de vida. Estos resultados sugieren que el desarrollo de las uniones sinápticas en el núcleo es inversamente proporcional al peso del cráneo.

Various types of interactions between cholinergic neurons and the nigrostriatal DA system exist in the adult rat. The prenatal development of cholinergic influences on these DA neurons was first investigated with nicotine. This drug increases the firing rate of nigral DA neuron processes of 0.1-0.25 mg per kg. DA nerve cells become detectable by fluorescence histochemistry in the mesencephalic flexure of rat fetuses at embryonic day (ED) 13 (ED 1 = 24 h after mating). Their projections reach the neostriatum at ED 15-16.

Nicotine (0.4 mg/kg s.c.) was administered to the mother rat at ED 18 3/4 or 19 3/4. At this developmental stage the catecholamine fiber innervation of the neostriatum is fairly dense but shows the immature patchy pattern. The fetal brains were dissected 1-40 min after drug injection. DA was determined in midbrain and caudate-putamen by radioenzymatic assay. DA concentrations were markedly elevated in both regions after nicotine. Preliminary experiments revealed an influence of the drug also after direct injection into the fetus. These observations demonstrate that fetal nigrostriatal DA neurons are responsive to nicotine. However, the biochemical characteristics of the reaction differ from the adult state where DA concentrations remained unchanged in the somatodendritic and terminal areas. Parallel changes in the neonatal rats point to differences between pre- and postnatal animals in the nigral response to nicotine. Biochemical and histochemical observations on fetuses in the last third of gestation indicate that the nigrostriatal DA system is also influenced by chronic nicotine treatment during pregnancy. The biochemical aspects of these drug effects are further investigated.

Our results show that nicotine affects a prenatal central catecholamine system, the nigrostriatal DA neurons. A direct assessment of drug effects in the fetal period may contribute to an understanding of the relationship between prenatal influences and their possible consequences for postnatal life.


The concept of a refractory period of responsiveness to ether stress in the neonatal rat has been questioned. Since the original determination of this stress non-responsive period had been based on the relatively insensitive fluorometric assay of corticosterone (B), we decided to re-evaluate this phenomenon using the more sensitive technique of competitive protein binding assay. Serum and adrenal B were measured 15 min after exposure to a 1-min ether (E) stress, as well as to subcutaneous injections of ACTH (A, 20 I.U./100 g b.w.) and saline (S) in rats 5, 7, 9, 11, 13, 15, 20, and 25 days of age. Furthermore, the time course of the response was determined in 25-day-old rat pups 0, 1, 3, 5, 10, 15, 30, and 60 min following exposure to E. Significant elevations in serum and adrenal concentrations of B were measured at all ages following the treatments; the serum B values (ug/100 ml) are shown in the table:

<table>
<thead>
<tr>
<th>AGE</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>.33</td>
<td>.91</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>E</td>
<td>.91</td>
<td>1.1</td>
<td>1.7</td>
<td>2.6</td>
<td>3.3</td>
<td>10.5</td>
<td>8.6</td>
<td>6.6</td>
</tr>
<tr>
<td>A</td>
<td>1.3</td>
<td>.92</td>
<td>1.9</td>
<td>3.1</td>
<td>3.5</td>
<td>2.2</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>S</td>
<td>1.3</td>
<td>.81</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The serum and adrenal response to E was maximal by 15 min in the 5-day-old animal. In the 15-day-old rat serum response continued to rise thru 60 min, whereas the adrenal response became maximal at 15 min. We concluded that the neonatal rat is capable of a significant response to stress as early as the age of 3 days and that this response is independent of all age studied. Furthermore, the 9-day-old rat does not show a delayed response to stress as evidenced by the significant rise in corticosterone levels observed 15 min after ether.
THE HALTHERE-LIKE PROJECTION OF SEGMENTALLY TRANSLOCATED WING RECEPTORS IN DROSOPHILA HOMOLOCUS MUTANTS. Derek Schubiger* and John Palka. Dept. Zoology, Univ. Washington, Seattle, WA 98195. When sets of sensory cells are experimentally moved from one location to another, their axons often assume a distribution which places the c.n.s. in a characteristic and invariable place of origin, when the ganglion cells of an inverted amphibian eye project to the tectum according to their original orientation relative to the body. Certain receptors on the wing of Drosophila act in this way when their axons are caused to enter the c.n.s. in the metamorphic segment by the movement of a group of receptor neurons. In silver-intensified cobalt fills we have not been able to detect any differences in the distribution of the receptor fibers in flies with and without normal external morphology is clearly of wing type. A more wing-like projection might be obtained from these flies if the normal metathoracic wings were removed prior to the time when sensory fibers reach the c.n.s., thus leaving a substrate vacant and ready for occupancy by the homotopic wing fibers. We have produced wingless flies both genetically and by using a wingless kit of stocks, and surgically by extirpating one wing disc in bithorax prepupae. In silver-intensified cobalt fills we have not been able to detect any differences in the course of the homotopic fibers in flies with and without normal wings. Thus, an occupied wing substrate does not appear to be responsible for the haltere-like course taken by the axons of the small campaniform sensilla whose axons, instead of going "home" to the metathorax, follow the paths of the haltere receptors they have replaced. The wings and halteres of wild type flies both carry small campaniform sensilla. Their morphologies are similar but not identical and their central processes are likewise very close to each other in many places but are not overlapping; in certain locations they are entirely distinct. The small campaniform sensilla of halteres homotopically translocated into wings follow the path of wild type haltere fibers in every respect we have examined, even though they come from wing tissue and their external morphology is clearly of wing type. A more wing-like projection might be obtained from these fibers if the normal metathoracic wings were removed prior to the time when sensory fibers reach the c.n.s., thus leaving a substrate vacant and ready for occupancy by the homotopic wing fibers. We have produced wingless flies both genetically by using a wingless kit of stocks, and surgically by extirpating one wing disc in bithorax prepupae. In silver-intensified cobalt fills we have not been able to detect any differences in the course of the homotopic fibers in flies with and without normal wings. Thus, an occupied wing substrate does not appear to be responsible for the haltere-like course taken by the axons of the small campaniform sensilla of the homotopic wing. PSYCHOPHARMACOLOGICAL EFFECTS OF APOMORPHINE DURING ONTOGENY. Ismail A. Shalaby* and Linda Patia Spear. Dept. Psychology, SUNY-Binghamton, Binghamton, N.Y. 13901. Behavioral time-sampling methods were used to assess the ontogenetic patterns of psychopharmacological responsiveness to the dopaminergic agonist, apomorphine. Male and female Sprague-Dawley rats were given 0, 0.05, 0.1, 1.0 or 3.0 mg/kg apomorphine hydrochloride and tested for changes in matrix crossing behavior (forepaw crossing against wall) and the number of matrix crossings. At these ages, only slight amounts of sniffing behavior were seen even after the highest doses of apomorphine. By P28, the number of matrix crossings decreased at all doses of apomorphine (e.g., Matthe et al., Br. Res. 136 (1977, 319). Also at P28, administration of either 1 or 3 mg/kg apomorphine increased matrix crossing behavior and induced marked stereotyped sniffing, gnawing, or licking behavior. Apomorphine-induced wall climbing behavior was seen only during the first two postnatal weeks. We have also observed that the α-noradrenergic agonist clonidine similarly stimulated wall climbing behavior only during this postnatal interval, although the noradrenergic reuptake inhibitor cocaine did not induce wall climbing at any age (Sprague-Dawley, in press). Moreover, wall climbing can also be induced by footshock (Fish, personal communication) or wall shock (Stethouer & Campbell, Develop. Psychobiol., 1979, in press). Possible explanations of these similarities in insult-induced behavior at early postnatal ages, and their subsequent decline during ontogeny, will be discussed. DEVELOPMENTAL SPECIFICATION OF COLUMNAR AND RETINOTOPIC STRUCTURE OF RETINO-GENICULO-STRIATE SYSTEM VIA A MOVING BOUNDARY VALUE DIFFUSION-DECAY DECOY SYSTEM. A Schwartz & E L. Schwartz (SPON: E. R. John) Brain Research, Dept. of Psychiatry N.Y.U. In previous work, it has been shown that the pattern of afferent input to striate cortex may be described in terms of a concatenated complex logarithmic mapping (Schwartz, 1977a), and that the retinotopic map is generated in generations of brainstem nuclei, as described in terms of solutions to a variational problem in which the magnitude of the magnification factor is minimized, subject to prescribed boundary conditions imposed by the visual and cortical anatomy (Schwartz, 1977b). In the present work, it is shown that the moving boundary value diffusion-decay system that has recently been described as a possible mechanism for the modified Lindenmeyer-Veen equation possesses the correct spatial structure to describe the receptive field density of L/M direction columns on a local scale, as well as retinocortical homogeneity on a global scale. Remapping this spatial pattern to the cortex via the variational principle proposed in Schwartz(1977b) results in an accurate model of local and global retino-ridge-ridge striate functional architecture. Ocular dominance and orientation column structure are provided with the correct relative column scale factors, and it is shown that the ising model allows an analytic study of the (phase) transition from overlapped to columnar ocular dominance pattern. Finally, a general variational principle for fiber sorting in models most other models as special cases, and which is implemented by a convenient computer algorithm which may be used to solve the general decaying system. This work indicates that details of retino-geniculo-ridge map development may be simulated quantitatively and analytically, and that the variational principle provides a theoretical approach to developmental specification(Schwartz,1977b) may provide a comprehensive description of retinotopic formation. Schwartz, E.I. Bio. Cybernetics 28: 1-14(1977a) Schwartz, E.I. J. Theol. Bio. 69:65-66(1977b) Lindenmeyer, A. and Veen, A. Plant Physiology 50:127-134 (1977) DEVELOPMENT OF PIONEER AND SENSORY AFFERENT PROJECTIONS IN THE GRASSHOPPER EMBRYO. Warty Shansky* (SPON: B. Bergin) Neurobiology Group, Univ. Calif., Berkeley, Ca. 94720. The embryonic formation of a sensory projection will be described in the grasshopper, Schistocerca gregaria. Cobalt diffusion staining (silver intensified) of the cereal nerve depics sequential ingrowth and central branching of both pioneer and sensory axons. Pioneer neurons (Bate, ’76, Nature 260: 54-56; Edwards and Chen, Roux’s Arch. Dev. Biol., in press) in the cereal lumen establish the neural pathway to the sensory afferents and the neuropil is restricted to pioneer axons which form the central commissures linked by two bilateral longitudinal fiber tracts (LFT). At least five pioneer axons are seen in the cereal nerve. They enter the LFT at its junction with the most posterior commissure and form numerous fine processes. Two or three thicker axons that end in growth cones travel several segments anterior as part of the LFT. These axons do not branch, but bear short, fine processes which later disappear. The four posterior ganglion rudiments fuse at 60% of embryogenesis. Sensory axons enter this fused ganglion at 63% and obscure the projection, but the pioneer neurons still persist. Unbranched, multisegmental longitudinal axons are present even in the adult. Furthermore, cobalt fill into the hatching cercus stumps of normal cells, but also into a pair of most other models within the lumen (pioneers) whose dendrites do not contact sensilla. Hence, at least some pioneer neurons survive after embryogenesis. The pioneers have been described as pathfinders, leading the way, and also as a pair of most other models within the lumen (pioneers) whose dendrites do not contact sensilla. Hence, at least some pioneer neurons survive after embryogenesis. Sensory axons enter this fused ganglion at 63% and obscure the projection, but the pioneer neurons still persist. Unbranched, multisegmental longitudinal axons are present even in the adult. Furthermore, cobalt fill into the hatching cercus stumps of normal cells, but also into a pair of most other models within the lumen (pioneers) whose dendrites do not contact sensilla. 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GUIDANCE AND TOPOGRAPHIC PATTERNING OF RETINAL GANGLION CELL AXONS.


In order to examine the factors that may guide the earliest developing optic axons in mice, the stretch of primitive neuroepithelium located along the potential route of the pioneer optic nerve fibers has been examined in three dimensions with the use of computer graphic reconstruction of serial, 1 µm sections. The study has revealed a system of wide-bored intercellular channels (averaging about 25 µm² in cross sectional area) within the marginal zone of the primitive retina and continuing without interruption into the underside of the optic stalk (adjacent to the optic fissure) and toward the presumptive optic chiasma. The spaces appear well in advance of the morphological differentiation of the retinal ganglion cell neurons.

The openings are arranged with a definite directionalality. Thus, the spaces at the region of the prospective optic disc appear to coalesce and form segregated sets of long, interconnecting tunnels oriented in the direction of the stalk. More peripherally, toward the back and rim of the cup, the spaces form blind, radially arranged pockets. Previous studies have established that the first developing retinal ganglion cells with axons are generated immediately dorsal to the optic stalk (Mann, '66; Kahn, '75), that is, at the junction between the open tunnels and closed pockets. Given the constraints of their extracellular surroundings it is conceivable that the earliest optic axons may be confronted with alternative environments depending on their direction of movement and may literally be compelled to exit the eye in the proper direction. Indeed, the path taken by the first outgrowing optic fibers is identical to the one previously established by the intercellular tunnels and always in the direction of the stalk.

Over their entire course, the tunnels in the region of the disc usually maintain their positions in relation to the optic fissure and, thus, particular segments of the retinax become connected by continuous openings with equivalent regions in the stalk. As the later forming optic axons (those near the back and periphery of the cup) add on sequentially, perhaps by fasciculation, they are never given the option of growing towards the pupil since the pockets interposed between them and the rim of the cup always remain closed. Providing that the first optic axons approach the disc in register, the layout of these specialized extracellular spaces may not only provide directional information to the first forming optic axons but topographic cues as well.

Supported by grants from NINCDS and American Cancer Society.
Box 590: MORPHOLOGY OF RETINOTECTAL CONNECTIONS IN FETAL MOUSE EXPLANATE CO-CULTURES

Electrophysiological studies of retinotectal co-cultures (Smalheiser and Crain, Br. Res., 494:100-106, 1988) have now been extended by horseradish peroxidase (HRP)- and cholera toxin subunit B (CTb)-mediated axon tracing experiments. HRP injections into the retina of 18-20 day old mouse fetuses allowed for the visualization of axons entering the tectum and emanating from the ganglion cell layer. The axons emerged from the optic chiasm and entered the tectum, where they formed a dense network of fibres. The axons terminated in the superficial layers of the tectum, indicating the presence of functional retinotectal connections.

Box 591: LATE DEVELOPMENT OF DENDRITIC STRUCTURE IN N. LAMINARIS

The late development of dendritic structure in the superior colliculus (NC) of the mouse was studied using both postnatal and adult specimens. The dendritic structure of NC cells was characterized by a gradual increase in complexity from postnatal day 10 to adulthood. The dendritic trees became more horizontally oriented, and the complexity of individual dendrites increased over time. The development of dendritic spines was observed, and these spines showed a significant increase in density during the postnatal period.

Box 592: KINETICS OF $^{22}$Na AND $^{36}$Cl DISTRIBUTION IN THE POST- NATAL RAT CHOROID Plexus

The kinetics of $^{22}$Na and $^{36}$Cl distribution in the postnatal rat choroid plexus was studied using a radiotracer technique. The uptake of $^{22}$Na and $^{36}$Cl by the choroid plexus was found to be dependent on the age of the animal. The uptake of $^{22}$Na was found to be higher than that of $^{36}$Cl, indicating a higher permeability of the choroid plexus to sodium ions.

Box 593: DEVELOPMENT OF HIPOTHALAMIC LHRH AND SERUM LH, FSH AND PROLACTIN IN INTACT AND NEONATALLY ADRENALIZED MALE AND FEMALE RATS AND NEONATALLY ANDROGENIZED FEMALE RATS

The development of hypothalamic LHRH and serum LH, FSH and prolactin was studied in intact and castrated male and female rats. The results showed that the concentrations of these hormones increased with age. The peak concentrations were reached at around 25 days of age in intact animals, and at day 10 in castrated animals. The androgenized females showed a significant increase in LHRH content until day 25, and the concentrations of FSH and LH were significantly higher in intact males compared to females.

Despite differences in serum FSH concentration between intact and androgenized animals, the increase in serum FSH levels in intact animals was not evident until day 10 in female rats and after day 25 in male rats. Androgenized females did not demonstrate an increase in serum FSH content over the period of observation. This increase in hypothalamic LHRH might be related to the tonic destruction of estrogen or possibly, to the high concentration of prolactin occurring after puberty in the androgenized animals.
PHYSIOLOGICAL DEVELOPMENT AND SEGMENTAL DIFFERENCES OF NEURONS FROM AN IDENTIFIED PRECURSOR DURING GRASSHOPPER EMBRYOGENESIS. N.C. Spitzer, M. Bate*, and C.S. Goodman. Dept. of Biology, UCSD, La Jolla, CA, 92038, and Max-Planck-Institut fdr Virusforschung, Tübingen, F.D.R.

The "H" neuron, one of the two progeny of the midline precursor cell 3 (P3p) in the grasshopper, undergoes a morphological transformation early in the first instar. During this transformation in the metathoracic ganglion it acquires many of the same physiological properties as the first progeny of the DUM neuroblast in the same ganglion, at the same time that the latter cells are differentiation from the "H" lineage. The transformation of the P3p takes place while still electrically coupled to its mitotic sibling. The onset of electrical excitability is first seen in the new processes and shows thereafter that the cell body becomes electrically excitable. In those cases in which the sibling persists, its axon but not its cell body becomes electrically excitable.

Although all of the segmental ganglia come from relatively similar sets of embryonic precursor cells, there are striking differences in the number and properties of the neurons in the mature ganglia. In the meso- and metathoracic ganglia (T2 & T3), one of the two progeny of P3p is transformed into the "H" cell. In contrast, none of the abundance the fourth (A4), both P3p progeny disappear, at the time of the transformation of the "H" cell in T2 and T3. A gradient of transformation and cell death is seen in the first thoracic ganglia (A2), at the boundary between thorax and abdomen. From A1 to A3 the P3p progeny acquire fewer of the morphological properties of the "H" cell: they lose the original process less often, and extend fewer of the new axons. There is also variability in the apparent gradient of the number of progeny persisting from A1 to A3. Finally, the acquisition of only one of the morphological phenotypes is paralleled by the partial acquisition of the physiological phenotypes: the progeny of P3p in A1 to A3 develop excitable axons but do not develop the soma spikes seen in the progeny of different precursor cells in the same segment can develop similar phenotypes.

(Distributed by the NIH, NSF, Helen Hay Whitney Foundation, and Max-Planck Gesellschaft.)
Synaptogenesis in the Developing Antennal Lobe of the Brain of the Moth Manduca sexta.  Leslie P. Tolbert.  Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The antennae and antennal lobes of the brain of the moth Manduca sexta arise during the 18 days of metamorphic adult development, and therefore provide a convenient preparation in which to study the developmental interactions between neurons that will form a mature synaptically active brain. A basic understanding of the correlation between the neurochemical and cytological maturation of the antennal lobes, we have examined the protein composition of adrenergic and cholinergic cells when grown in media conditioned by certain non-neuronal cells, where they develop into mature, differentiable proteins involved in the formation and function of these two types of synapses. Synaptic transmission in the antennal lobe is found in the plasma membrane, in the extracellular matrix, in synaptic vesicles, and as soluble proteins free to diffuse between cells. To separate and characterize the transmitter-specific proteins, we have analyzed each of these subclasses of neuronal proteins separately: (1) Membrane glycoproteins were labeled either metabolically, by incorporation of [3H]thymidine into the cell membrane, or oxidatively with periodate or galactose oxidase, followed by reduction with [3H]BH4. Both methods labeled a similar group of proteins. (2) Soluble proteins were secreted and/or shed into the culture medium under normal growth conditions. These were also labeled metabolically by incorporation of [3H]leucine. (3) Electron micrographs were taken on one dimensional (SDD) and two dimensional (isoelectric focusing/SDS) gel electrophoresis and autoradiography. Data will be presented that shows that each of these subclasses of cellular proteins is distinct. Each contains from five to over ten different polypeptides. It has been possible to identify adrenergic and cholinergic-specific proteins in each subclass, and a preliminary characterization of these will be described. Determination of neuronal transmitter choice thus involves not only the enzymes required for transmitter synthesis, but also a number of other proteins which are extra-cellular or exposed on the surface of the cell. (Supported by a NINDS grant to P.H. Patterson and a NINDS Postdoctoral Fellowship to K.J.S.)

Patterns of Neurogenesis in the Ventral Lateral Geniculate Nucleus of the Chick.  C. J. Uchwat* and W. J. Crossland, Dept. of Anatomy, Wayne State University School of Medicine, Detroit, Mich. 48201.

This study was designed to determine the temporal and spatial patterns of neurogenesis in the chick ventral lateral geniculate nucleus (GLv). A single injection of 20 μCi of [3H]-thymidine was applied to chick embryos and the time course of appearance of PNMT in vitro is similar to that in vivo. The cultured embryonic adrenals may serve as a useful model for the analysis of the factors involved in the initiation of PNMT synthesis. (Supported by N.I.H. Grants DH 18974 and NS 03346.)
ALTERED ISTHMO-TECTAL TOPOGRAPHY AFTER EYE ROTATION IN XENOPUS

In normal Xenopus, the tectum receives a direct visuospatial input from the contralateral eye and an indirect input from the ipsilateral eye. Both projections arise from the contralateral tectum; in turn, each tectum projects to its ipsilateral nucleus isthmi (NI) and the NI then projects across the midline to the tectum of the contralateral side. Hence, each tectum projects from the two eyes are in register with each other; each point in the binocular visual field is represented at a single point in each tectum via these projections.

If one eye is rotated during mid-larval stages, each point on the retina still projects to its appropriate site in the contralateral tectum, i.e. the projection map of the visuotectal tectum is rotated. The indirect ipsilateral visuotectal map from the normal eye develops in such a way that the two visuotectal projections are in register, i.e., the indirect projection from the normal eye becomes rotated. On the other tectum, the normal eye still produces a normally oriented direct visuotectal map, and again, the indirect ipsilateral projection is in register, i.e., the rotated eye produces a normally oriented ipsilateral projection. We have studied the tecto-isthmo-tectal pathway in an attempt to reveal whether this plasticity comes about by means of rearrangements of the terminals of the crossed isthmo-tectal projection.

At this hypothesized rostral level, we have made localized horseradish peroxidase (HRP) injections in the tectum to determine the topographic relationship between the tectum and the NI in normal and eye-rotated frogs. The results show that each tectal HRP injection labels a discrete group of tecto-isthmic fibers in the ipsilateral NI. This tecto-isthmic topography appears to be the same in normal and eye-rotated frogs. In normal frogs, each injection also labels a discrete group of cells in the contralateral NI. In eye-rotated animals, however, a single HRP injection usually labels two groups of cells in the contralateral NI. One group corresponds to the contralateral tectum, the other consists of cells which normally would be labelled by an HRP injection in a different tectal site. We interpret our results to mean that a new population of crossed isthmo-tectal cells is arborizing at the injection site and that the original population is labelled because they are either passing through their original positions on route to new sites or are forming physiologically inactive arbors at their original sites. Thus, it appears that the anomalous topography of the indirect ipsilateral projection results from alterations in the termination sites of the crossed isthmo-tectal fibers.

SUPPORTED IN PART BY NIH GRANT EY05211 TO S.B.U.


Sympathetic neurons taken from superior cervical ganglia of neonatal rats and maintained in cell culture can develop either adrenergically or cholinergically depending on culture conditions. Both cholinergic deafferentation and electrical activity can stabilize the choice of adrenergic development, even in the presence of the cholinergic inducing factor secreted by certain neuronal cells. Both CA3 and CA4 elevated cAMP, which may be the coupling factor. Depolarization elevates neuronal cAMP content, and exposure to dibutyryl cAMP or forskolin, which elevate cAMP, also stabilize the adrenergic choice, suggesting that cAMP may be the second messenger.

To clarify the role of CA3 and cAMP, some of their interrelations were studied. When adenurines were depolarized in the presence of Mg2+ or D600, no increase in cAMP was seen. On the other hand, BTA, dibutyryl adenosine, and theophylline did not alter the elevation in cAMP but did reverse the developmental effect of depolarization. Thus, CA3 appears to be more directly involved in the depolarization effect, since manipulating its cellular distribution can uncouple the cAMP increase from stabilization of the adrenergic choice. Mg2+ and D600 are probably more efficient than the other agents at blocking Ca2+ influx through the voltage-dependent channel. The developmental effect on cAMP could indicate that the increase is in response to this type of Ca2+ entry. α and β adrenergic blockers and adenosine blockers both influence cAMP release, indicating that these substances is not an intermediate step.

Restricting CA3 availability with BTA also reversed the effect of dibutyryl cAMP on transmitter choice. Furthermore, neurons exposed to adenosine, PGE2, or dibutyryl cAMP in the presence of BTA responded with a normal increase in cAMP but failed to select a new adrenergic pathway. These results support the interpretation that these agents are acting by increasing neuronal Ca2+, though the possibility remains that CA3 and cAMP act directly at a later step controlling Ca2+ entry. In summary, the CA3 and cAMP systems of these neurons appear to be multiply interconnected, but CA3 appears to be more directly linked with the choice of transmitter choice. (Supported by an NINDS grant to Paul Patterson.)


Androgen exposure during fetal life produces changes characteristic of the masculine phenotype in mice as evidenced from developmental anomalies and the administration of exogenous androgen. In sharp contrast, a novel approach to the study of sexual differentiation has involved the examination of a naturally occurring phenomenon—sexual differentiation in the normal male. Specifically, female mice that develop in utero between the normal VN organ and the deafferented VN organ are capable of mating with a male fetus (OM females) differ in a variety of characteristics: for example, OM females are more sexually attractive to males, have longer estrous cycles, are less responsive to male pheromones, and are more likely to become sexually quiescent when grouped; 2M females, on the other hand, are more aggressive in a variety of situations and have larger gonadal structures at birth. It was hypothesized that androgens provided by a fetus might be transferred to an adjacent female fetus via the amniotic fluid and thus be present in higher concentrations in both the amniotic fluid and blood of a 2M female relative to an OM female. To test this hypothesis, mice fetuses were delivered on Day 17. Aminolithic fluid was collected on filter paper, and blood serum was taken from 7M and 7S OM females. Testosterone, progesterone, and 17α-estradiol were measured by radioimmunoassay after chromatographic separation.

Naturally higher levels of testosterone were detected in the serum and amniotic fluid of 2M females (serum: 2M females = 1,090 ± 46 pg/ml; OM females = 878 ± 43 pg/ml; t = 2.7, p < 0.05, df=8; amniotic fluid: 2M females = 128 ± 2 pg/ml; OM females = 102 ± 6 pg/fetus; t = 4.0, p < 0.01). Estradiol and progesterone levels in amniotic fluid and serum did not differ (p > 0.1). The results suggest that the hypophysis in the hypophysis of OM females are capable of responding to androgens provided by a male fetus via the amniotic fluid and thus be present in higher concentrations in both the amniotic fluid and blood of a 2M female relative to an OM female. To test this hypothesis, mice fetuses were delivered on Day 17. Aminolithic fluid was collected on filter paper, and blood serum was taken from 7M and 7S OM females. Testosterone, progesterone, and 17α-estradiol were measured by radioimmunoassay after chromatographic separation.

FURTHER OBSERVATIONS ON POSTNATAL NEUROGENESIS IN THE SNAKE'S VOMERONASAL EPITHELIUM: USE OF 3H-TYTHIDINE AUTORADIOGRAPHY TO TRACE THE DIFFERENTIATION, MATURATION AND MOVEMENT OF BIPOLAR NEURONS. R.J. Wang, A. Vayagovitk, B. Nemelsohn* and M. Halpern, Dept. of Anatomy Cell Biology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

The vomeronasal epithelium of adult garter snakes is capable of postnatal neurogenesis. The findings from previous studies involving the vomeronasal organ at each survival time, labeled cells which had been tagged by 3H-thymidine injection without being subjected to the lesion-making procedure. The heads of all animals were processed for light microscopic autoradiography. Application of this technique to analyze the differentiation and sexual attractiveness. The implications of the present findings are that the point on a continuum of these characteristics that a female occupies is correlated with serum levels of testosterone during prenatal development.

Supporting information for Medical Research, Mill Hill, London NW7 1AA, England.

DEVELOPMENT


TRACE THE DIFFERENTIATION, MATURATION AND MOVEMENT OF BIPOLAR NEURONS. R.J. Wang, A. Vayagovitk, B. Nemelsohn* and M. Halpern, Dept. of Anatomy Cell Biology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

The vomeronasal organ is the organ at each survival time, labeled cells which had been tagged by 3H-thymidine injection without being subjected to the lesion-making procedure. The heads of all animals were processed for light microscopic autoradiography. Application of this technique to analyze the differentiation and sexual attractiveness. The implications of the present findings are that the point on a continuum of these characteristics that a female occupies is correlated with serum levels of testosterone during prenatal development.

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Supporting information for Medical Research, Mill Hill, London NW7 1AA, England.

Catecholamine agonists and antagonists can have lasting effects on the developing brain. Since L-DOPA is used in treating cerebral palsy, and since cAMP and/or catecholamines monitor the rate of cell division in many systems, we investigated the relationship between L-DOPA treatment and brain DNA synthesis in immature rats. L-DOPA (30 to 500 mg/Kg) inhibited DNA synthesis in forebrain in a dose-dependent fashion. A DOPA-decarboxylase inhibitor (RO4-4692, 800 mg/Kg), which raises brain L-DOPA but not catecholamines, blocked the inhibition of DNA synthesis by L-DOPA, while monoamine oxidase inhibitors potentiated this effect. This inhibition was maximal in the first 10 days of life in forebrain, but was not manifest until 10 days in cerebellum, suggesting that it depended on the stage of maturation of the tissue. In vitro, norepinephrine and dopamine in concentrations sufficient to elevate cAMP (0.1 to 1 nM) and dibutyryl cyclic AMP inhibited DNA synthesis in brain slices. These results suggest that therapeutic amounts of L-DOPA can alter brain DNA synthesis and presumably brain development; whether this effect is mediated by catecholamines and cAMP requires further investigation. Until this hypothesis is ruled out, the possibility of adverse effects on development should be kept in mind when using neurotransmitter precursors, agonists or antagonists in the neonate.

Supported by Research Grant NS 13515 from NIMH and by the Research Service of the Veteran’s Administration.

THE DEVELOPMENT OF β-ADRENERGIC RESPONSIVENESS IN CNS MOUSE REAGGREGATE CULTURES J. M. Wehner* and J. R. Sheppard, University of Minnesota Dight Institute for Human Genetics, 400 Church Street S.E., Minneapolis, MN 55455, U.S.A.

Mouse CNS reaggregate cultures were prepared using fetuses after 17 days of gestation. Whole fetal brains were removed and mechanically dissociated into single cells as described by Honneggar and Richelson (Brain Res. 109:338, 1976), and maintained in GM1 + 15X horse serum. The development of β-adrenergic hormone responsiveness was examined as a function of time in culture. The time course of cAMP synthesis (using intact reaggregates) in response to 10^{-7} Isop. revealed that a major peak of cAMP production (after 10 min of hormone exposure) appeared at 11-13 DIV (equivalent to 8-10 days after birth). This corresponds to the development of catecholamine response in cerebral cortex described by Harden et al. (Brain Res. 125:99, 1977). The stimulation of cAMP production was dependent upon the dose of Isop. (10^{-9} to 10^{-13} M), and was blocked by the addition of β-propranolol. Adenylyl cyclase activity was also measured using homogenates prepared from reaggregates at 4, 14, 21 DIV. Basal and fluoride-stimulated activity increased as a function of time in culture. Isop. (10μM) or GTP (50μM) did not stimulate adenylyl cyclase activity in homogenates of 4DIV reaggregates. However, adenylyl cyclase activity was stimulated 2-3 fold by Isop. or GTP and 7-8 fold by Isop. + GTP at 14 & 21 DIV. These experiments indicate that β-adrenergic responsiveness appears in reaggregate CNS cultures at approximately the same time that such a response appears in vivo. (Supported by NIH Grant NS-14435.)
A GOLGI ANALYSIS OF EARLY DIFFERENTIATION OF MOTOR NUCLEI IN THE HINDBRAIN OF THE MOUSE EMBRYO. Leo E. Weidman, Dept. Anat., Sch. Med., Univ. of California, San Francisco 94143

To this date no one has described the very early differentiation of mammalian cranial nerves using the Golgi technique. Last year, using a modified Golgi technique described (Anat. Rec., 190: 580-581) neuronal differentiation throughout the CNS in a mouse embryo of ten days gestation (E10; E0=gestational plug). Cells from younger and older embryos have been successfully prepared and fixed, but neuronal differentiation has not been observed since then. At E9-9 1/2 weeks of hypoglossal neurons exit in a direct line with the ventral roots of cervical nerve. By contrast, all cells of the entire cranial complex, facial motor nucleus, and trigeminal motor nucleus exit at the level of the neural lamina. Their cell bodies are located in a position similar to that of spinal accessory neurons at this age, i.e., ventromedial to somatic efferent neurons, e.g., the hypoglossal nucleus. The bulbar portion of C.N. XI and the vagus nerve appear to be part of a continuous complex at this age. A few of the cells of the trigeminal motor nucleus have started to migrate towards their existing root. By E10 the hypoglossal nucleus is all developed and these cells are becoming multipolar. The majority of the cells of the nucleus ambiguous complex still reside ventral to hypoglossal neurons. Occasionally a cell is seen located ventral to the exiting root of the vagus. These cells may be early cells of the dorsal motor nucleus of the vagus. A row of motor neurons of the origin of the vagus nerve extends along the lateral border of the neural tube from a most ventral position to almost the level of exit of the motor root. Although many of the ventral cells around the root are primarily arranged with the bases which have migrated closer to the exiting root have reached the bipolar stage. By E11 some cells of the X-XII nucleus have migrated so far medially that the axons of the cells which have migrated closer to the exiting root have reached the cephalic bladder. 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Since the chick embryo offers numerous advantages for developmental studies, we are attempting to establish a model system to explore the interactions between noradrenergic (NA) neurons and their target cells during brain development in this experimental animal. As investigated by histofluorescence, the locus coeruleus (LC) has been reported to project to the cerebellar cortex of the adult chicken (Mugnaini and Dahl, J. Comp. Neurol. 162 (4):417, 1975). In the present study we have confirmed this report using the horseradish peroxidase tracing technique. In the rat LC-cerebellar system, the time of origin (last cell division) of LC cells precedes that of cerebellar Purkinje cells by several days (Lauder and Bloom, J. Comp. Neurol. 155 (4): 469, 1974), a pattern we have now examined in the chick. The amount of 3H-thymidine (3H-T) (s. a. 57 Ci/mMol) available to the embryo at various developmental stages following a single injection (25 µCi) into the yolk sac at 1-2 days of incubation (d. i.) was determined by thin layer chromatography of acetic acid extracted yolk, white or embryonic brain tissue. Contrary to previous assumptions, results of this study indicate that 3H-T is not continuously available to the chick embryo after a single injection, but rather constitutes a 1-2 day pulse. This finding permits the use of long-survival 3H-T autoradiography in chicks to date the time of last cell division within a 2 day range using the adult brain rather than that of the embryo, greatly facilitating accurate identification of cell populations in discrete brain regions. Therefore, we have used this method to determine the time of last cell division of LC cells and cerebellar Purkinje cells in the chick. A single dose (25 µCi) of 3H-T was injected into the yolk sac of fertilized eggs on day 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 or 8 d. i. Chicks were sacrificed between 29 and 44 days posthatching and the brains prepared for autoradiography. Cell proliferation ceased in the chick LC from 2-6 d. i., with a peak of heavy labeling between 3-5 d. i., whereas this event occurred in the Purkinje cell precursor population from 3-7 d. i. with a peak of heavy labeling between 4-6 d. i., indicating that the chick LC begins to develop prior to the cerebellar Purkinje cells in a manner similar to that observed in the rat. This finding, together with our data confirming a specific LC-cerebellar projection in the chick, provides the basis for the use of this system as a model for examining NA neuron target cell interactions during avian embryonic development. Supported by U of Connecticut Research Foundation.


In view of the anticipated increased use of atomic energy in industry, in continuation of our previous work (Radiation Res. 72, 117, 1979) we studied the possible long term effect of chronic radiation exposure. Rats were given tritiated drinking water (3µCi/ml) before pregnancy, during pregnancy and thereafter, continuously through 5 generations (F1 to F5); females were mated with control males. The average total daily intake was 5µCi. At this dose, no signs of radiation illness were observed. In each generation, 30 day old male and female pups were sacrificed and their brains assessed for accumulation of radioactivity and for development.

Radioactivity significantly increased through generations: as compared with F1, the radioactivity in F5, and subsequent generations was up to 1365 higher in the blood and up to 88% higher in the cerebral cortex than in the cerebellum (209%). The highest specific activity was in the cortical DNA fraction (up to 85000cpm/mg); in the protein fractions the highest specific activity was in the dienecephalon (up to 2000 cpm/mg) and brain stem (up to 1780 cpm/mg).

The damages to the developing brain were also significantly intensified through generations: as compared to the control, the decrease in DNA (cell number) and protein in F5 was 3 to 4 times more severe than in F1. The most severe decreases were: DNA (cell number) of brain stem (-31%), dienecephalon (-25%), cerebral cortex (-23%) and cerebellum (-18%); protein of dienecephalon (-32%), brain stem (-30%), cerebral cortex (-28%) and cerebellum (-27%); all these differences were statistically highly significant (p<0.001); on the average for both DNA and protein they were 1½ times more severe in dienecephalon than in the cortex. These damages at 30 days were also 2 to 3 times higher than in the newborns.

Several phenomena may account for all these results. Postnatally proliferating cells (glia in cerebral cortex, most neurons and glia in cerebellum) may be more sensitive to radiation damage than the prenatally proliferating neurons. DNA involved in protein synthesis (mostly postnatal) is repaired less efficiently (if at all) than the prenatally proliferating neuronal DNA. (Supported by DOE grant ET-76-S-03-0034 and NIH grants HD-05615 and Al-00162).
EPILEPSY
DIFFERENTIAL EFFECTS OF ANTICONVULSANT DRUGS ON CORTICAL- AND ALDRAHILIZED SEIZURES IN THE RAT. Larry J. Bearden and W. W. Burnham. Dept. of Pharmacology, Univ. of Toronto, Toronto, Ontario, Canada.

Previous studies involving the kindling model have indicated that seizures kindled from the amygdala respond differentially to therapeutic drugs. The present study was designed to gain further insight into the pharmacology of these seizures. Specific anticonvulsants were given intraperitoneally (i.p.) or orally (p.o.) to rats with established amygdala-kindled seizures. The anticonvulsants were evaluated for their ability to suppress EEG and behavioral changes associated with amygdala-kindled seizures. The anticonvulsants tested included dipropylacetate, progabide, ethosuximide, valproic acid, and sodium valproate. The effects of these drugs were evaluated on two components of the seizures: 1) the local generalization component and 2) the partial and generalized seizures. The anticonvulsants did not reverse the effects of dipropylacetate, progabide, or valproic acid, but ethosuximide and sodium valproate showed some degree of anticonvulsant activity. These results suggest that amygdala-kindled seizures may have a different pharmacology from other types of seizures.

Analysis of the EEG and behavioral changes associated with amygdala-kindled seizures revealed that the anticonvulsants had a variable effect on the different components of the seizures. However, the most consistent effect was the suppression of the hypersynchronous waves (300-500 μV, 3-6 Hz) coinciding with a behavioral depression. This pattern is quickly followed by the onset of quiescence. The anticonvulsants tested did not prevent the suppression of the hypersynchronous waves, but they did alter the subsequent behavioral depression. The anticonvulsants were most effective in suppressing the hypersynchronous waves and behavioral depression, but they were less effective in preventing the subsequent behavioral depression. These results indicate that the pharmacological profile of amygdala-kindled seizures may be different from other types of seizures.

PROCAINE-INDUCED SEIZURES IN MONKEYS WITH BILATERAL HIPPOCAMPAL AMYGDALA-INDUCED SEIZURES. Larry J. Bearden and W. W. Burnham. Dept. of Pharmacology, Univ. of Toronto, Toronto, Ontario, Canada.

Intravenous injections of local anesthetics at low doses have been shown to depress central nervous system functions (Livingston and Perrin, 1972; Julien, 1973; Demetreacu and Jolicoeur, 1974) and have been recommended for the treatment of status epilepticus (Bernhard and Bohn, 1955; French et al., 1975). However, at higher doses a variety of cocaine derivatives produce seizures in man (Julien, 1973; Munson et al., 1969) and man (de Jong and Walts, 1966). These results have suggested that cocaine-like drugs may selectively activate limbic epileptogenic areas without activation of generalized tonic-clonic seizures.

Chronic focal epilepsy can be produced in rats by subcutaneous injection of ferric chloride. Ferric chloride is a potent convulsant that produces seizures in rats. However, these seizures are not identical to spontaneous psychomotor seizures, and the effects of ferric chloride are not specific to limbic structures. The present study was designed to determine the effects of ferric chloride on the structure and function of the brain. Ferric chloride was given intraperitoneally to rats for 14 days. The rats were then evaluated for their response to electrical and chemical stimulation. The results of this study suggest that ferric chloride may selectively activate limbic epileptogenic areas without activation of generalized tonic-clonic seizures.

The results of this study indicate that amygdala-kindled seizures may have a different pharmacology from other types of seizures. The anticonvulsants tested in this study did not prevent the suppression of the hypersynchronous waves, but they did alter the subsequent behavioral depression. These results suggest that amygdala-kindled seizures may have a different pharmacological profile from other types of seizures.

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**ROLE OF FOREBRAIN CATECHOLAMINES IN AMYGDALOID KINDLING.**


Previous evidence has suggested that central catecholamines play some role in kindling, a permanent increase in susceptibility to seizures after intermittent electrical stimulation of the brain. Specifically, it has been reported that amygdaloid kindling was facilitated by intraventricular injections of 6-hydroxydopamine (6-OHDA) that produced marked depletion of noradrenaline (NA) and dopaminergic fibers distributed throughout the brain. The present study was designed to determine whether NA, DA, and their agonists had similar effects on kindling.

Depletion of NA and DA was produced by bilateral intracerebral injections of 6-OHDA. Animals were divided into four groups: (1) sham injections, (2) vehicle injections, (3) bilateral 6-OHDA injections, and (4) bilateral 6-OHDA injections followed by vehicle injections. The threshold intensity of stimulation for the development of an epileptic discharge was determined in each group. The results demonstrated that bilateral 6-OHDA injections significantly increased the threshold intensity of stimulation for kindling.

Subsequent experiments were designed to determine whether the observed increase in threshold intensity of stimulation was due to a direct effect of NA depletion on the amygdala or to the development of a compensatory mechanism. These experiments revealed that bilateral 6-OHDA injections did not affect the threshold intensity of stimulation for kindling when the injections were made in the absence of any drug. However, when the injections were made in the presence of a sympathomimetic drug, such as isoproterenol, the threshold intensity of stimulation was significantly increased. This effect was not observed when the injections were made in the absence of a drug that would inhibit NA reuptake, such as desipramine.

These results suggest that the development of a compensatory mechanism following NA depletion is responsible for the increased threshold intensity of stimulation for kindling. This mechanism may involve the release of catecholamines from other brain regions that are responsive to NA depletion.

**EFFECTS OF CORTICAL EPILEPTOGENIC ACTIVITY ON DEVELOPMENT OF RECEPTIVE FIELD PROPERTIES IN THE VISUAL CORTEX OF YOUNG RABBITS.**


A previous study (Chow, et al., Brain Res., 146: 151, 1978) showed that normal development of receptive field properties in the lateral geniculate nucleus occurred within the normal period of development. This study was designed to determine whether the development of receptive field properties was affected by chronic epileptogenic activity in the visual cortex.

The visual cortex of paralyzed, anesthetized cats was studied in vivo before and after the injection of Na-penicillin. The results showed that the normal development of receptive field properties was disrupted during chronic epileptogenic activity. This disruption was observed within 10 minutes of the injection of Na-penicillin and persisted for at least 24 hours. The effects of chronic epileptogenic activity were not observed in the visual cortex of normal, intact cats.

These results suggest that the development of receptive field properties is disrupted by chronic epileptogenic activity in the visual cortex.
KAINIC ACID ELICITS ELECTROGRAPHIC EPILEPTIFORM ACTIVITY AFTER CENTRAL AND PARENTERAL ADMINISTRATION TO AWAKE RATS. L.C. Crawford and W.G. Wooten. VA Epilepsy Ctr. and Dept. Neurology and Pharmacology, Univ. Texas Health Sci. Ctr. at Dallas, TX 75216.

Kainic acid, a toxic compound, is known to produce toxic brain edema and enhance neuronal excitability. We have investigated the effects of kainic acid (KA) on central and peripheral neuronal excitability in awake rats and determined the minimum effective dose (MED) for KA. These experiments were conducted to determine the safety of central and peripheral administration of kainic acid in awake rats.

Intracerebroventricular (icv) and intraperitoneal (i.p.) injections of KA were administered to awake rats. The rats were observed for 24 hours post-administration and their behavioral responses were recorded. The MED for KA was determined by administering KA in increasing doses to awake rats and observing their behavioral responses. The MED for KA was found to be 2 mg/kg for icv administration and 10 mg/kg for i.p. administration. These results suggest that KA is a potent neuronal excitant in awake rats and that the MED for KA is lower in icv administration compared to i.p. administration.

Epileptic seizures were observed in awake rats following icv and i.p. injections of KA. The seizures were characterized by tonic-clonic movements, convulsions, and loss of balance. The seizures lasted for up to 30 minutes and were followed by a period of recovery. These results suggest that KA is a potent epileptogenic agent in awake rats and that the MED for KA is lower in icv administration compared to i.p. administration.

The effects of KA on awake rats were further investigated by administering KA in increasing doses to awake rats and observing their behavioral responses. The MED for KA was determined to be 2 mg/kg for icv administration and 10 mg/kg for i.p. administration. These results suggest that KA is a potent neuronal excitant in awake rats and that the MED for KA is lower in icv administration compared to i.p. administration.

The results of these experiments suggest that KA is a potent neuronal excitant in awake rats and that the MED for KA is lower in icv administration compared to i.p. administration. These results also suggest that KA is a potent epileptogenic agent in awake rats and that the MED for KA is lower in icv administration compared to i.p. administration.

Supported by the NIMH and the Veterans Administration.

TRIMETHADIONE AND SEIZURE SUSCEPTIBILITY IN EPILEPTIC FOWL. N.L. Pavlew, D.D. Johnson and R.C. Crawford. Dept. of Pharmacology and Animal and Poultry Science, Univ. of Sask., Saskatoon, Saskatchewan, Canada S7N 0W0.

Trimethadione (TMO) and diethylthiobutyramide (DMO) were administered to chickens. The chickens were tested for seizure susceptibility before and after the administration of TMO and DMO. The results suggest that TMO and DMO are effective in preventing seizures in chickens.

The chickens were tested for seizure susceptibility before and after the administration of TMO and DMO. The chickens were found to be more susceptible to seizures after the administration of TMO and DMO. The results suggest that TMO and DMO are effective in preventing seizures in chickens.
EXPERIMENTS ON HIPPOCAMPAL “KINDLING” IN THE RABBIT. C.L. Ehlers*, P.C. Chamber**, D.I. Whitey** and C.H. Saywer. Dept. Anath. and Brain Res. Research, LCCA, College of Medicine, CA 20708

The process of “kindling” is a phenomenon in which a progressive increase in neural and behavioral responsiveness is produced by a spaced and repeated epileptogenic stimulation of specific brain sites. In the present study we investigated the development of kindling following electrical stimulation of the dorsal hippocampal complex of the rabbit. To modify kindled electroencephalographic and behavioral responses, five New Zealand White rabbits were stereotaxically implanted with concentric bipolar electrodes aimed at two sites in the hippocampal and one in the amygdala (AMY), as well as screws placed in the calvaria over frontal and limbic cortices (CTX). The kindling stimulus (1 m sec biphasic square wave pulses, 60 Hz frequency, 200 μA current, 1 sec train) was applied once daily until 3 fully-kindled convulsions (chewing, rearing, falling) were noted. The initial stimulations produced only brief behavioral arrest and hippocampal afterdischarge (AD) which did not spread to CTX or AMY. However, with repeated stimulations a sequences of “psychomotor” behaviors emerged which included sniffing, darting of the head from side to side, facial twitching, wet dog shakes, posturing, grooming of the face and violent kindling thumbing associated with urination (X = 11.6 days). During this time (X = 10.4 days) independent interictal discharges in DHPC appeared which were enhanced during rest and slow-wave sleep, and were blunted during attention. With repeated stimulation AD was seen to spread to AMY and frontal CTX; following this the animals began to show typical rhythmic chewing characteristic of AMY kindling (X = 31.2 days). Animals produced similar seizures soon thereafter (X = 29.0 days), although they remained “partial” in nature, i.e., the chewing and roaring were ipecacuivisive to the following kindled behavioral patterns. After the third kindling stimulus these responses appeared different. Single doses of pheno­barbital (30 mg/kg IP), carbamazepine (20 mg/kg PO) and Diazepam (5 mg/kg IP) all attenuated or blocked fully-kindled responses, although some of the “psychomotor” behaviors remained. Chlorpromazine (10 mg/kg IM) had no effect on fully-kindled responses, although it did block the “psychomotor” behavior of the kindled rat. None of the drugs blocked independent interictal discharges in DHPC. (Supported by NIH, the Ford Foundation and the Gianinni Foundation.)


It has been suggested that symptoms of Huntington’s Disease (HD) may partially result from the excitotoxic action of glutamate, which is a known neurotransmitter in HD. Evidence occurring in the intracaudate administration of KA is a behavioral index of the excitotoxic action of KA. Conceivably agents capable of reducing such excitotoxic action might be considered as potential treatments for HD. Tauroine is a naturally occurring inhibitory aminoacid (AA), 3 amino propane sulfonic acid (APS) is a powerful excitotoxic analog that systemic administration can enhance epileptic discharges (Fariello, Exp Neu in press). In the present study we have tested the effects of these inhibitory AA on the behavioral changes and the seizures induced by bilateral intracaudate injection of KA. Two groups of rats were pretreated for four days with IP APS or Taurine (500 mg/kg/day). These two groups plus a third non pretreated group were then injected with 1 ul of 4.7 mM KA in both caudate nucleus. Behavior and seizures were then continuously monitored in all animals for 6 hours. At 26 and 48 hrs after injections animals were sacrificed and their brains histologically examined. The mean number of seizures during the 6 hrs post injection are reported below for each group.

Control KA 94
APS 73
Taurine 36

In the taurine group seizures were also of less severity and shorter duration. Hyperactivity, excessive grooming, jumping and wet dog shaking behavior were more prominent in the 3 APS group, followed by KA and only moderately present in taurine rats. Taurine seems to offer significant protection against the epileptogenic and hyperactivity inducing effects of KA. The results are further discussed in light of the histological findings in the various groups at different times after KA administration.

631 NEOCORTICAL EPILEPTOGENESIS IS NOT REDUCED BY TRANSVERSE CORtical LESIONS: JOHN W. FERGUSON and Howard Williams. Div. of Neurology, Case Western Reserve U. Sch. of Med. and Cleveland VA Hospital, Cleveland, Ohio 44106

In our previous studies we indicate that the responses of reticular formation (RF) neurons to sensory stimuli are greatly enhanced by subconvulsant doses of pentylentetrazol. This suggests that the convulson does not produce enhancement by acting predominantly on sensory receptors. Pentylentetrazol at 3.139, 1977) but found ADT was significantly elevated (0.43±.05 ma) compared to non-lesioned quadrants (0.17±.02 ma, p .001). 2. In cats with 4 parallel TCLs in one SS and 4 TCLs in an adjacent quadrant, and in animals with 4 TCLs forming a box in one quadrant, a slight ADT elevation in the lesioned quadrants was not significant compared to non-lesioned quadrants but both were signifi­cantly elevated compared to controls. (F.0.32 ± 0.10, 1.03 ±.03 ma vs 0.13±.06 ma). 3. ADD as in previous experiments with surface stimulation, was elevated in lesioned quadrants compared to non-lesioned. Grouping all lesioned animals, it was signifi­cantly elevated in lesioned (13±5±2.23 sec) and non-lesioned (10±6±3.3 sec) quadrants in lesioned animals showed no change and are not time dependent within the 40 days. Sectioning horizontal cortico-cortical connections affects epileptiform activity in non-lesioned cortical areas. Although some support for the hypothesis is gained, epileptogenesis may be enhanced because of these lesions as shown by prolonged duration of afterdischarge.

ON THE SITE OF CONVULSANT-INDUCED ENHANCEMENT OF SENSORY RESPONSES OF RETICULAR NEURONS: IONTOPHORETIC STUDIES. C.L. Faingold, W.K. Hoffman* and D.M. Coteley. Divisions of Pharmacology and Neurobiology, Dept. Medical Sciences, Southern Illinois University School of Medicine, Springfield, IL 62708

In order to examine the site of convulsant-induced enhancement of sensory responses of reticular neurons, we have performed iontophoretic application of strychnine (40-380 nA) or PTZ (65-190 nA). Following application of strychnine, the enhanced responses of RF neuronal firing was often lasting for up to 30 min. However, the repetitive spike bursting pattern observed with systemic administration of strychnine (Faingold and Stittworth, Neurosci. Abs. 3:139, 1977), while mesencephalic RF neuronal firing is increased to a similar extent of RF response enhancement. The effects of microiontophoresis on primary sensory neurons are not sufficient to explain the extent of RF response enhancement. In some cases the enhancement of RF neuronal firing by convulsion could be reversed by iontophoresis of the inhibitory amino acids glycine and GABA. Since iontophoresis of convulsants produces RF response enhancement, a major portion of the effect of systemic convulsant on RF neuronal responses may be exerted directly on the reticular formation. These data further support the theory that actions of convulsants on the reticular formation is important to the development of drug-induced generalized seizures. (Supported in part by S.I.T. Foundation.)

Although invertebrate glial cells appear to respond to changes in extracellular potassium concentration, \( [k^+]_o \), in a manner predicted by the Nernst equation, variable results have been obtained in studies of the response of mammalian glial cells to \( [k^+]_o \) changes (Somjen, 1975; Futamachi and Pedley, 1976). In feline neocortex we simultaneously recorded intraglial potentials and adjacent \( [k^+]_o \) changes induced by direct cortical stimulation (DCS). A series of stimulus trains, each train of greater intensity than the previous trains, was administered until after discharge (AD) was produced. We found that at the height of glial depolarization following DCS, the slope of the relationship between glial depolarization and \( [k^+]_o \) changes was 52.9 mV membrane potential change for a 10-fold change in \( [k^+]_o \). During AD, however, the relationship between glial depolarization and \( [k^+]_o \) changed significantly. AD associated glial depolarization larger than that occurring during DCS was seen in only 3/14 instances despite \( [k^+]_o \) rises which invariably exceeded the levels reached during DCS. These observations suggest that glial depolarization is related to \( [k^+]_o \), in a manner approximating that predicted by the Nernst equation when measurements are made just after the termination of DCS, however during AD the relationship between glial depolarization and \( [k^+]_o \) changes. Factors accounting for this change in relationship will be discussed.


The Mongolian gerbil (Meriones unguiculatus) has been proposed as a model for the epilepsies (Loskota et al., Epilepsia 15:109, 1974) and stratum of seizure-sensitive and seizure-resistant gerbils have been established by several laboratories. Previous research has indicated structural differences between the two strata in CA3 zone of the hippocampus (Paul et al., Neurosci. Abs. 4:165, 1978). The main differences, as observed with the light microscope using Golgi techniques, were in the distribution of spines on CA3 pyramidal cells and in the shape and size of the mossy tufts of dentate granule axons. These changes have been now further studied with the electron microscope and several observations seem worthy of description. 1) Synaptic vesicles in mossy saccs in seizure-sensitive gerbils appear to be densely and homogeneously distributed throughout the tuft rather than clustered near a synaptic thickening as observed in seizure-resistant gerbils. We wonder whether the vesicle-crowded tuft in the seizure-proe gerbil may not reflect the disordered ictal state. 2) The seizure-sensitive gerbils exhibited evidence of ongoing degenerative changes in 3- and post-synaptic elements in CA3 and CA4 zones of the hippocampus. These changes appeared as electron dense profiles with most of the contained membranous components degenerated.

In view of similar degenerative changes observed in previous Golgi and electron microscope studies in temporal lobe epilepsy in man (Brown, UCLA Forum in Medical Sciences, 17:339, 1973; Scheibel et al., Epilepsia 15:55, 1974) further studies are underway to compare the structural changes in the latter disorder with similar changes in the convulsive gerbil.

DIPHENYLHYDANTOIN: INTERACTION WITH \([3H]Diazepam Binding Site in Brain and Anticonvulsant Activity. Dorothy W. Gallager, Pierre Hallgorge*, and John F. Tallman. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

Using extracellular recording and microiontophoretic techniques, the anticonvulsant agent diphenylhydantoin (DPH) was found to increase the efficacy of benzodiazepines when tested on dorsal raphe neurons in the anesthetized rat. This increased biological effect could be correlated with an enhanced specific binding of \([3H]Diazepam ([3H]DZ) in brain following pretreatment of rats with DPH. Enhanced \([3H]DZ binding could be demonstrated in an in vitro assay using cortical membranes from animals pretreated with DPH in vivo. In addition, using an in vivo labeling technique, DPH pretreatment significantly enhanced the amount of specifically bound \([3H]DZ in brain. Increased binding (of approximately 15%) when measured at 0.25 mM \([3H]DZ following treatment of rats with DPH is due to an increase in the affinity of the brain-specific benzodiazepine receptor for its ligand. However, this enhanced binding does not appear to be a direct effect of DPH on \([3H]DZ binding or due to an increase in brain GABA levels. Increases in \([3H]DZ binding were found to be dose-dependent following anticonvulsant doses of DPH, while not significantly altered by subconvulsant doses of DPH. In developing rats, effects on brain-specific \([3H]DZ binding were found to parallel the time course for the development of the anticonvulsant activity of DPH. In rats younger than 3 weeks, treatment with DPH resulted in non-significant decreases in \([3H]DZ binding, while in rats 3 weeks of age and older, significant increases in \([3H]DZ binding were seen as compared to vehicle-injected littermates. Since DPH, the benzodiazepines and several other agents which cause alterations in benzodiazepine binding site affinity are active as anticonvulsants, the data suggests that the benzodiazepine binding site in brain may be involved in some of the anti-convulsant properties of these drugs.

Although the occurrence of homocysteine-induced behavioral seizures in animals has been reported in the literature, the effect of this model in the study of experimental epilepsy has not been fully exploited. We believe that this model has theoretical and practical advantages over many of the currently used models of experimental epilepsy. For example, homocysteine is present in the brain in both homocysteine and homocysteine, but unlike MSO, homocysteine occurs endogenously as a metabolite of methionine, and some patients with elevated plasma levels of homocysteine have had seizures.

With i.p. injections of homocysteine, dose-related electrographic and behavioral seizures are observed. The seizures are reproducible and can be visualized. Doses of 2-6 mg/kg/active latent coalt foci in rats. Focal spiking in the electrocorticogram (ECoG) is observed. At doses of 3 mg/kg/active limbic seizures is observed in synchrony with the ECoG during spasm. At higher doses, electrographic and behavioral seizures are also seen in unlesioned animals. Doses of 5 mg/kg/active (ED50) is associated with clonic limb movements. The seizures recur within 30 minutes.

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We have further studied the effects of homocysteine on rats in mice. A major pathway for homocysteine metabolism occurs via the methionine synthetase reaction, which requires N5-methyl tetrahydrofolate (N5MTHFA) and vitamin B12. Pretreatment of animals with N5MTHFA (1 mg/kg), tetrahydrofolate (4 mg/kg) or methotrexate (4 mg/kg) decreased the latency of homocysteine-induced seizures (p < .05). Chronic pretreatment with vitamin B12 (25 mg/kg per day) increased the latency and decreased the severity of seizures.

These results have a clinical correlate, as recent reports have indicated a decrease in the number of seizures when epileptics were administered a combination of folate plus B12 rather than folate alone in treating the metabolic complications of anticonvulsant therapy. Prophylaxis was also associated with a split-litter technique. Along with the ECoG during spasm, the seizures recur within 30 minutes.

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PERSISTENT INCREASE IN HIPPOCAMPAL NEURONAL EXCITABILITY PRODUCED
by REPEATED INJECTION OF PENTETETRATRIZOL. A. O. Oliver, N. Hoffer, and R. J. Wyatt. Laboratory of Clinical Psychopharmacol., DSMR, New England Hospital, Washington, D. C. 20032.

Repetitive injections of chemical convulsants are reported to induce progressive lowering of seizure thresholds, a phenomenon known as "kindling". Epileptogenic activity was studied in guinea pig hippocampal slices to examine the physiological characteristics which may be involved. Intercital spike (IIS) frequency was used as the primary parameter. Slices from normal guinea pigs, perfused with 95% O2/5% CO2, were incubated in a physiological chamber which was adjusted to equilibrated solutions of 5000 U/ml penicillin. The configurations of the IIS were also similar to the two groups. The kindled animals convulsed about 80% of the time following PTZ injection. However, spontaneous convulsions were not observed during drug-free periods in the kindled group. The data suggest that the kindled group is more resistant to the effects of these convulsants.


Extending work previously begun in our laboratory, we have investigated possible structural correlates of seizure behavior in the Mongolian gerbil (Meriones unguiculatus). A seizure-susceptible (SS) and a seizure-resistant (SR) strain have been selectively bred in the laboratory of Dr. R. Schain. Investigations were performed using histochemical, histological, and electron microscope techniques. Quantitative analysis of Golgi-imregnated material using improved tissue-blocking and staining methods enabled a clear visualization of hippocampal CA1 pyramidal cells before and after seizures. The number of spines per CA1 pyramidal cell was reduced in the kindled animals compared to the normal control group. The data suggest that changes in the structure of the hippocampus may be involved in the development of seizure susceptibility.

MEAN SPINE COUNTS (Number of spines/45 µ dendritic segment)

Hippocampal Pyramidal Cells

SS (n=19) 88.1 ± 7.0
Basilar 73.8 75.5 ± 6.0
Apical 73.8 74.2 ± 6.0

n=number of cells

The loss of spines in CA1 may indicate only that we are not observing a generalized metabolic deficiency. Results from transmission electron microscopy reported elsewhere at this meeting indicate differences between SS and SR animals in several parameters. Structural variations in mossy fiber terminals implicating on these pyramids have also been noted. We are examining the brains of developing animals by histological and electron-microscopic methods to determine the extent of these changes before and after seizures. The data suggest that changes in the structure of the hippocampus may be involved in the development of seizure susceptibility.

Figure 1 illustrates BAEP changes seen in the cat during administration of enflurane at a dose producing bisynchronous spike-waves. There is an increase in I-V interpeak latency of 8 standard deviations. There was no significant change in interpeak latency with administration of equipotent doses of all non-epileptogenic anesthetics studied to date: halothane, thiopental, nitrous oxide, or isoflurane (the isomer of enflurane).

In the monkey, there is a significant increase in I-V interpeak latency, as shown in Figure 2, with administration of pentetetrazol in doses producing an electro-clinical model of petit mal epilepsy.

Addition to the animal data, there is evidence for BAEP alterations during spike-wave bursts in patients with "absence" seizures. For one of the patients seen (Bost, there was an increase in over 2 standard deviations in I-V interpeak latency during spike-wave activity. In another patient (Rochester) induction of 3 per second spike-wave discharges with photic stimulation was associated with highly significant transitory prolongation of I-V interpeak latency that persisted for a short period after the spike-wave activity.

In summary, there seems to be alterations in functioning at the level of the rostral brainstem, as indicated by BAEP changes, during generalized spike-wave activity induced by drugs in animals, and accompanying absence seizures in humans.
HALOTHANE ANESTHESIA AND THE ANIMALS WERE PARTIALLY IMMOLIZED.

LIDOCAIN AND SALINE TREATED ANIMALS WERE PREPARED AND STUDIED IN AN EXPERIMENTAL MODEL FOR TEMPORAL LOBE EPILEPSY.

THE INCREASE IN AVERAGE GLUCOSE UTILIZATION WAS OBSERVED IN HIPPOCAMPUS, DENTATE GYRUS, AMYGDALA, SEPTAL NUCLEI, AND ENTORHINAL CORTEX. THE INVERSE CORRELATIONS BETWEEN METABOLIC RATE AND DURATION OF SEIZURES FOLLOWED BY INTERMITTENT CLONIC MOVEMENTS OF HEAD, TRUNK, AND FOREPAWS; STRAUB TAIL PHENOMENON; AND REARING AND FALLING.

THE CURRENT STUDY THE C-2-DEOXYGLUCOSE METHOD WAS USED TO DETERMINE THE EFFECTS OF A REPEATED MASSED STIMULATION PARADIGM ON KINDELING. THE INTERVAL BETWEEN EACH KINDLING STIMULUS AND THE PRESENTATION OF MASSED STIMULATIONS HAS BEEN SHOWN TO HAVE AN IMPORTANT EFFECT ON KINDLING. IN THIS STUDY, A GROUPED TRIAL SESSION (GTS) CONSISTING OF 5 DAILY STIMULATIONS, ONE HOUR APART, WAS USED TO DETERMINE THE EFFECTS OF A REPEATED MASSED STIMULATION PARADIGM ON KINDLING. ALL RATS WERE STIMULATED ELECTRICALLY IN THE RIGHT ANGRENDA WITH A 60 CPS, 400 µM STIMULUS OF 1 SEC. DURATION.

SEIZURE INTENSITY WAS DETERMINED BY AFTERSHOCK (AD) DURATION AND BEHAVIORAL RESPONSE (BR) CLASSIFICATIONS CHARACTERISTIC OF KINDLED SEIZURES IN RATS. THE EFFECTS OF REPEATED GTS SESSIONS ON KINDLED RATS WERE DETERMINED IN GROUP I BY PRESENTING 6 CONSECUTIVE DAYS OF GTS TO RATS PREVIOUSLY KINDLED BY ONE STIMULATION PER DAY. THESE ANIMALS HAD KINDLED NORMALY WITH CONSISTENT AD'S AND BR'S WHEN STIMULATED ONCE DAILY, BUT HAD REDUCED AD'S AND INCONSISTENT BR'S DURING THE GTS DAYS. WITHIN 5 DAYS OF RETURNING TO DAILY STIMULATIONS ALL ANIMALS (GROUP I)responded with PRE-GTS SEIZURE INTENSITY. THE EFFECTS OF GTS ON THE DEVELOPMENT OF KINDLING WERE TESTED BY STIMULATING THE ANIMALS IN GROUP 11 USING ONLY THE GTS PARADIGM. THESE ANIMALS NEVER HAD THE CONSISTENT AD DURATION OR BR CHARACTERISTIC OF RATS KINDLED WITH JUST ONE STIMULATION PER DAY.

FOR COMPARISON, GROUP III RATS WERE STIMULATED ONCE DAILY FOR 32 HOURS DURING WHICH THE AD DURATION INCREASED NORMALLY, BUT BR'S WERE INCONSISTENT WITH ONLY 4 OF 9 ANIMALS SHOWING CLINICAL MOTOR SEIZURES. WHEN REDUCED TO DAILY STIMULATIONS, THE GROUP AD AND BR RESPONSE WAS GREATLY DIMINISHED COMPARED TO THE HOURSLY STIMULATION PERIOD, BUT ALL ANIMALS HAD CONSISTENT KINDELING DURING THE 32 HOURS. THESE FINDINGS SUGGEST THAT A PROLONGED INHIBITION OF KINDLED SEIZURES RESULT FROM REPEATED MASSED STIMULATION AND THAT ANIMALS KINDLED WITH REPEATEDLY MIGHT NOT SHARE ALL THE NEUROPHYSIOLOGICAL CHARACTERISTICS OF THOSE KINDLED WITH STIMULATIONS ONCE A DAY.

THE ILLUMINATION OF THE VARIOUS PHYSIOLOGICAL AND BEHAVIORAL CHANGES OCCURRING DURING SEIZURES IN THE LIMBIC SYSTEMS WAS TESTED. IN ANIMALS WITH LIMBIC STRIATUM SEIZURES, THE CURRENT STUDY THE C-2-DEOXYGLUCOSE METHOD WAS USED TO MEASURE LOCAL CEREBRAL METABOLIC RATES AND TO IDENTIFY NEURAL SYSTEMS INVOLVED IN THE PROGRESSIVE KINDELING EFFECTS ON BEHAVIOR AND DEVELOPMENT OF SEIZURES. TWENTY-ONE MALE SPRAUGE-DAWLEY RATS WEIGHING 250-300 GM. WERE ADMINISTERED LIDOCAINE (65 MG/KG, I.P.) OR SALINE ONCE DAILY (5 TIMES A WEEK) FOR 12 TO 43 DAYS. ELEVEN OF THE 13 LIDOCAIN-TREATED ANIMALS EVENTUALLY EXHIBITED ONE OR MORE SEIZURES (MAXIMUM: 28, MEAN: 9) LIVING SEVERAL SECONDS TO AS LONG AS 30 MINUTES WITH SALIVATION, CHEWING, AND PERIORAL MOVEMENTS FOLLOWED BY INTERMITTENT CLINIC MOVEMENTS OF HEAD, TRUNK, AND FOREPAWS; STRAUB TAIL PHENOMENON; AND REARING AND FALLING. LIDOCAIN AND SALINE TREATED ANIMALS WERE PREPARED AND STUDIED IN PAIRS. ONE FEMORAL ARTERY AND VENOUS WERE CATHETERIZED UNDER LIGHT HALOTHANE ANESTHESIA AND THE ANIMALS WERE PARTIALLY IMMOBILIZED IN LOOSE FITTING PELVIC PLASTER CASTS AND ALLOWED TO RECOVER AT LEAST 2 HOURS FROM ANESTHESIA. THE ANIMALS WERE THEN INJECTED WITH LIDOCAIN OR SALINE I.P., AND APPARENTLY 10 MINUTES LATER THE MEASUREMENT OF LOCAL GLUCOSE CONSUMPTION WAS CONSISTENTLY PERFORMED ON 3 DEOXYGLUCOSE (C-2-DEG) CARRIED OUT ON 20 NEURONS (J. NEUROCHEM. 1975; 28: 897). COMPARED TO CHRONIC SALINE TREATED CONTROLS, ALL LIDOCAIN-TREATED ANIMALS HAD 15-25% LOWER RATES OF METABOLIC ACTIVITY IN ALL AREAS OF BRAIN INCLUDING CORTEX, STRIATUM, BRAINSTEM, AND CEREBELLUM. IN ANIMALS WITHOUT OR WITH MILD SEIZURES LIDOCAIN TREATMENT ALSO REDUCED THE METABOLIC RATE THROUGHOUT LIMBIC STRUCTURES. IN ANIMALS WITH BRING PROLONGED SEIZURES, HOWEVER, STRIKING INCREASES IN GLUCOSE UTILIZATION WERE OBSERVED IN HIPPOCAMPS, DENTATE GYRUS, AMYGDALA, SEPTAL NUCLEI, AND ENTORHINAL CORTEX. THE INCREASES IN AVERAGE GLUTAMINE UTILIZATION OVER THE 45 MINUTE PERIOD OF MEASUREMENT CORRELATED POSITIVELY WITH THE DURATION OF SEIZURES.

THE ELECTROPHYSIOLOGY OF HUMAN EPILEPTIC NEURONS WAS EXAMINED BY PRINCE AND WANG (1977). THEY FOUND THAT DEPOLARIZATION EPSPs WERE CAPABLE OF GENERATING FOCAL EPILEPTIFORM ACTIVITIES. TWENTY OF 22 NEURONS FROM SUCH CORTEX GENERATED BURSTS OF 3-7 SPIKES WITH ORTHODROMIC STIMULATION. INTRACELLULAR RECORDINGS REVEALED THAT DEPOLARIZING POTENTIALS WITH AMPLITUDES OF UP TO 25 MV OR MORE AND DURATIONS LONGER THAN 100 MSEC WERE CAPABLE OF GENERATING SEIZURES.

THE ELECTROPHYSIOLOGY OF HUMAN EPILEPTIC NEURONS WAS EXAMINED BY PRINCE AND WANG (1977). THEY FOUND THAT DEPOLARIZATION EPSPs WERE CAPABLE OF GENERATING FOCAL EPILEPTIFORM ACTIVITIES. TWENTY OF 22 NEURONS FROM SUCH CORTEX GENERATED BURSTS OF 3-7 SPIKES WITH ORTHODROMIC STIMULATION. INTRACELLULAR RECORDINGS REVEALED THAT DEPOLARIZING POTENTIALS WITH AMPLITUDES OF UP TO 25 MV OR MORE AND DURATIONS LONGER THAN 100 MSEC WERE CAPABLE OF GENERATING SEIZURES.

DURING EXPERIMENTAL SEIZURES, INCREASED VASCULAR PERMEABILITY TO HORSE-radial PEROXIDE (HRP) OCCURS VIA ENHANCED VASCULAR ACTIVITY WITHIN THE ENDOTHELIAL CELLS OF SMALL PARENCHYMA VESSELS. IN ORDER TO DETERMINE THE RELATIVE CONTRIBUTION BY SMALL ARTERIES, ARTERIOLES, AND CAPILLARIES TO THE INCREASED VASCULAR PERMEABILITY, PINOCYTOTIC ACTIVITY WAS ASSESSED QUANTITATIVELY IN 6 PARALYZED, VENTILATED RATS WHOSE BLOOD PRESSURE AND ARTERIAL BLOOD GASES WERE MONITORED DURING THE EXPERIMENT. INTRAVASCULAR HRP WAS ALLOWED TO CIRCULATE FOR 5 MIN AND WAS FOLLOWED BY A SERIES OF 20 CONSECUTIVE ELECTROSHOCKS (100 MAMS, 1 SEC DURATION) EVERY 30 SEC. THE ANIMALS WERE ANESTHETIZED WITH Ether AND SUCCEDED BY PERFUSION-FIXATION WITH PARAFORMALDEHYDE-GLUTARALDEHYDE. A MID-CORONAL SECTION THROUGH THE THIRD VENTRICLE WAS INCUBATED FOR PEROXIDASE ACTIVITY AND PREPARED FOR LIGHT AND ELECTRON MICROSCOPY. LIGHT MICROSCOPY OF THE SERIAL 1UM SECTIONS FROM 3 ADDITIONAL ANIMALS SHOWED THE PRESENCE OF SEVERAL ARTERIES WITHIN EACH FOCUS OF HRP EXTENSION. ENDOTHELIAL CYTOPLASMIC AREA WAS DETERMINED FROM PLANIMETRY OF ELECTRON MICROGRAPHS TAKEN AT A CONSTANT MAGNIFICATION. THE NUMBER OF HRP-CONTAINING VESICLES PER 1MM2 ENDOTHELIAL CYTOPLASM WAS QUANTITATIVELY EVALUATED. MEAN PINOCYTOTIC ACTIVITY WITHIN THE ENDOTHELIAL CELLS OF THE DIFFERENT AREAS WAS: CAPILLARIES (211), 24.45 VESICLES/µM2; ARTERIOLES (37), 244.05 VESICLES/µM2. THESE RESULTS SHOW THAT SIGNIFICANT BLOOD-BRAIN BARRIER PERMEABILITY OCCURS WITHIN SMALL ARTERIES AS WELL AS CAPILLARIES, AND THAT THE SMALL ARTERIES MAY BE THE INITIAL SITE OF THE INCREASED VASCULAR PERMEABILITY OCCURS DURING SEIZURES.

Recent reports have demonstrated that both the di- and trivalent forms of iron are potent epileptogenic agents when in contact with the cerebral cortex. The present study was carried out to test the hypothesis that ionic iron released from hemoglobin plays a role in the development of seizures following intracortical bleeding. Adult Sprague-Dawley rats were anesthetized with sodium pentabarbital and secured in a stereotaxic apparatus. A 2mm hole was drilled through the skull over the left occipital cortex and with the aid of a micro-injection syringe fitted with a 30 gauge needle, 10 µl of a 13 mg solution of purified hemoglobin in saline injected 1.5 mm below the cortical surface. The animals were permitted to recover and maintained for periods of up to two months. EEGs were recorded, using needle scalp electrodes, daily for the first week and then weekly thereafter. All recordings were done under sodium pentabarbital anesthesia and continued until the animal showed signs of awakening. At varying intervals after hemoglobin injection animals were anesthetized and killed by transcardiac perfusion with neutral buffered formalin. Histological verification of the extent of the lesions was carried out. High amplitude focal spike activity with a frequency from 20 to 27 µsec spikes within 48 hours. The spike focus, when present, became relatively stable within two days after it was noted and in all cases persisted until the animal was sacrificed. No clinical manifestations of seizures were seen.

ASSOCIATED SLEEP DISTURBANCE IN CATS. M.B. Sternum, P. Hauri* and H.N. Shouse*. Sepulveda VA Med. Ctr., Sepulveda, CA and Depts. of Anatomy and Psychiatry, UCLA, Los Angeles, CA 90024

Lesions placed in ventrobasal thalami (VB) have been shown to reduce susceptibility to seizures both in man and in various experimental animal models of seizure disorders. In previous studies we have shown that such lesions protected cats against drug-induced seizures and facilitated sleep, which is typically disturbed with the development of seizures. In the present study the effect of VB thalamic lesions was examined in relation to chronic seizures induced by amygdaloid kindling and the disturbance of sleep which also accompanies the development of seizures with this model. Two groups of cats were prepared for basolateral amygdala kindling and standard sleep-waking state evaluation. Animals in both groups were monitored polygraphically during 12-hr recordings of EEG, EOG and EMG activity before and at intervals after daily stimulation of amygdala with a one-second train of biphasic square waves (1 msec pulse duration, 60 Hz, at currents just sufficient to elicit afterdischarge). After initial afterdischarge threshold were established animals in one group were subjected to bilateral electrolytic lesions of ventrobasal thalamus. Both groups were then studied until generalized tonic-clonic seizures were obtained with kindling stimulation. Animals in the non-lesioned group demonstrated kindled seizures were obtained with kindling stimulation or for a maximum of 75 days. Animals in the non-lesioned group demonstrated kindled seizures within 19-25 days and a progressive decrease in both slow wave and REM sleep. In contrast, animals with VB lesions required a minimum of 61 days for kindling or showed no generalized seizures within the 75 day period of study. Both groups of animals showed a disturbance of sleep during initial afterdischarge threshold determinations. This disruption of sleep was extended and sustained in animals without lesions during subsequent kindling but recovered completely within 30 days in animals with VB lesions. Animals in the latter group which failed to develop generalized seizures showed both remarkably stable afterdischarge thresholds and sleep state distributions. These findings support the body of data indicating that thalamic lesions protect against seizures. Further, they suggest that the conduction of abnormal discharge through sensorimotor thalamic circuits is involved in the propagation of seizure activity and the development of generalized seizure disorders of subcortical origin.

(Supported by the Veterans Administration)
Kindling refers to the progressive lowering of local afterdischarge (AD) thresholds and the progressive development of convulsive responses to initially innocuous electrical stimulation of limbic and other structures. It has been traditionally thought to reflect the breakdown, by repeated bombardment, of synaptic barriers between the site of stimulation and the motor systems. Studies of the epileptogenic effects of intraventricular injections of β-endorphin do not fit with this model, however. Injections of β-endorphin (10 µg) caused limbic epileptiform activity in rats which was not expressed in behavioral convulsions even when given to animals that had had their presumed limbic-motor synaptic barriers broken down by prior electrophysiological kindling. This was true whether amygdala, hippocampus, caudate, or dorsomedial thalamus was kindled to the stage of full clonic convulsions. The electrophysiological AD in the kindled hippocampus had greater amplitude than the ictal episodes caused by β-endorphin in kindled animals, but the biphasic pattern of response was the same; thus the two epileptiform events seem to have had qualitatively similar limbic effects. That the difference in amplitude might account for their differential effectiveness seems unlikely since the initial ictal episodes had greater amplitude than ADs at the beginning of kindling. That the amplitude of the ADs increased as a result of electrical kindling while the amplitude of the ictal episodes from β-endorphin did not indicates that there must be some difference between the two events which is more than a simple difference in amplitude. These data indicate that if synaptic barriers account for the failure of β-endorphin to cause behavioral convulsions in association with limbic ictal episodes, then the synaptic barriers involved must differ from those that block similar behavioral expression of ADs elicited by limbic electrical stimulation.
EVOKED POTENTIALS AND EEG

The application of Doyle's version (ECN 38:533, 1975) of a Wiener filter for averaged evoked potentials of the brainstem auditory evoked potential (BAEP) was evaluated as a means of reducing the number of stimulus-response pairs necessary to obtain a consistent representation of the BAEP. Averaged BAEP responses from 256 individual evoked potentials were processed using the Wiener filter and a simple narrowband filter (200-1600 Hz). This narrowband filter had previously been found to improve the identification of waves in the BAEP without distorting the waveform. The resulting waveforms were compared to both the unfiltered averaged potentials based on 256 individual potentials and an averaged potential from the same subject based on 2048 individual potentials. Subjectively, the Wiener filtered potentials were much more consistent from run to run than the narrowband filtered responses based on 256 individual potentials. The Wiener filter reduced the mean square error of the waveform, and the reduction was greater than that obtained with the narrowband filter. (The mean square error was estimated as the difference between the filtered wave and the averaged potential based on 2048 responses. The variability of latency and amplitude (peak-to-following trough) measures of waves I through V obtained from averaged potentials based on 256 individual responses was also reduced by the Wiener filter. Although the filtering variability was larger than the intersession variability of averaged potentials based on 2048 responses for a given subject, it was about the same for interobserver variability. The goodness of the spectral estimate of the evoked potential signal was observed to be an important factor in reducing the variability in the latency and amplitude data that has been discussed in the literature. Finally, the Wiener filter appeared to severely distort the waveform in many cases; although waves I through III were usually enhanced, wave V was often significantly reduced in amplitude. This problem appeared to be due to the magnitude of the signal spectral estimate in the frequency range of 200-300 Hz, but the cause of the problem has not yet been identified.


The field of GRS macro potential analysis is largely characterized by fragmented and unstandardized approaches to computer analysis. An impetus towards standardization has come from the clinical field with the development of the ACE test (Automated Cerebral Electrogram) which combines EEG and evoked potentials into a single procedure. Efficient processing of test data has been made possible by use of the MINICERAS computer system (LSI-11, 32K memory, 2 floppy disks, 100x sample A-D, X-Y and graphics printer output.) Apart from clinical uses this system has wide ranging research applications. The functions include: 1) spectral programs producing 16 channel spectral analysis displays (compressed spectral arrays) sampled over 3 minutes; 2) plots of spectral averages in the form curves for each electrode; 3) bin microvoltagges (delta, theta, alpha, beta, or selectable ranges) are generated and statistical programs provide significance tests for homologous electrodes and for normal populations matched for age; 4) area display programs make voltage contour plots of bin data (delta, etc.) or "time slices" of evoked potential fields at selected latencies following the stimulus; 5) recognition of patterns (i.e. spikes) is performed on 16 channels and the data assembled as "spike pack" graphics. By aligning primary spike data (recognized as "probable spike" by the computer algorithm) for final visual validation; 6) evoked potential averaging proceeds on 16 channels (brainstem response 4 channels) and computer programs recognize peaks and assign latency and amplitude automatically; 7) monitoring of EEG or evoked potentials over long periods and the construction of a "somogram" or "comagram" can be achieved. The system formats reports on the printographic terminal and can be used for data gathering, word processing, etc. Single unit processing algorithms are under development and customized software development will be available. Cost economy has been the major consideration in developing the system which is manufactured by Vision Systems of San Diego. (Supported by NIH Grant USPH/NS 08962-11.)
Computer Classification of Somatic Evoked Potentials Using Euclidean Distance and Cross Correlation. B. R. Martin, R. A. Matthews, and L. Martin. Department of Physiology, Medical University of South Carolina, Charleston, South Carolina 29403

Computer classification of somatic evoked potentials (SEP) obtained from monkeys subjected to different spinal cord lesions was compared to two different discriminant functions: cross correlation and Euclidean distance. Prototype SEPs were chosen arbitrarily from each lesion class. Each SEP was classified according to its relationship with the prototype. In the minimum distance classification procedure, a distance between the unknown and each prototype is calculated. The unknown is then assigned to the closest prototype, and each prototype is calculated. The unknown is then assigned to the closest prototype for which this coefficient is maximum.

In a series of experiments, monkeys were subjected to 3 different spinal cord lesions (anterolateral, hemisection, and central). Prior to the lesion three control SEPs were recorded from electrodes implanted in the skull overlying the upper limb and lower limb S1. A week after the lesion three SEPs were again recorded from each of these areas. For each lesion, the SEPs from the three different electrodes were used for computer classification. Two of the three recorded SEPs from each monkey were taken as prototypes from each class. The third SEP was classified. The classification programs were asked to assign each of 18 unknowns to one of four classes: normal, anterolateral section, hemisection, and central lesion. Both procedures classified over 90% of the unknowns correctly. The Euclidean distance classification made more correct classifications than the cross correlation program, indicating the significant importance of the unknown and each prototype is calculated. The unknown is then assigned to the closest prototype for which this coefficient is maximum.


Differences in tendencies to augment or reduce, as defined by the changes in activity or amplitude of visually evoked potentials (VEP) to increasing intensities of light stimulation (Lam & Segal, Science, 1974, 17, 1977) are believed to be related to frontal control of arousal. To test this hypothesis, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the augmenting/reducing pattern. In the first experiment, one group of rats received control of arousal, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the augmenting/reducing pattern. In the first experiment, one group of rats received control of arousal, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the augmenting/reducing pattern. In the first experiment, one group of rats received control of arousal, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the augmenting/reducing pattern. In the first experiment, one group of rats received control of arousal, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the augmenting/reducing pattern. In the first experiment, one group of rats received control of arousal, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the augmenting/reducing pattern. In the first experiment, one group of rats received control of arousal.

Three studies were conducted varying the environmental scale of subjects. The purpose was to assess changes in neurological functioning as postulated by a theory of experiential space-time relativity.

Environmental scale was manipulated three-dimensionally, two-dimensionally and perceptually through color. Each study involved a comparison between large and small scale conditions. In the first study scale contrast consisted of exposure to the full-size laboratory environment model replica of the space. In the second study, subjects viewed different image sizes of projected slides, the smaller being 1/3 the scale of the larger. In the third study scale contrast was provided perceptually by presentation of receding (cold) and approaching (warm) colors.

Neurological functioning was measured through absolute and percent power output of the left and right central cortex over seven EEG spectral bands comprising the range of 0-27 Hz. Electrodes were placed bilaterally over the motor cortex with active electrodes placed 10% and 20% measured from the vertex towards the ipsilateral ear with an ear-lobe ground. Data were analyzed according to a spectral program employing the Fast Fourier Transform.

Results of all three studies indicate two principal effects of reduced environmental scale on spectral EEG output: 1) an increase in absolute power output and a selective increase in percent power output across the higher frequencies (16-27 Hz).

These findings are consistent with the theory of experiential space-time relativity which postulates that the rather precise mediation of temporal experience by spatial scale (De Long, 1978, Technical University, 6:364-358) should exhibit neurological correlates. The selective increase in percent power output for higher frequency ranges seems compatible with the experience of time passing more swiftly for subjects under scale-reduced conditions. The increase in absolute power output across all spectral ranges still eludes interpretation, but appears consistent with reports by subjects that the compression of temporal intervals under scale-reduced conditions is experienced as being intense -- the temporal intervals feeling densely packed.

When considered in conjunction with the space-time theory, these findings suggest environmental scale may play a central role in mediating changes in neurological functioning associated with maturational development. Current studies are examining the potential role of environmental scale in maturational development and the etiology of hyperkinesis. (Research supported by the Office of Graduate Studies and Research, University of Tennessee)

There is growing acceptance that selective attention to task-related sensory stimuli elicits a subject's uncertainty eliciting cerebral evoked potential (EP) of 250-500 msec peak latency, namely the P300. The first section of this report examines somatosensory EPs by this method. Sensory evoked electrical potentials of four different fingers, where the subject is instructed to attend only to the number of stimuli delivered to one finger which has been designated the "target finger." Electrical stimulation of non-target fingers is to be ignored. Following the work of Desmedet et al. (1977), the authors have reported the finding that reliable P300 components may be obtained using this selective attention task. Robust P300 components were recorded over the central-parietal region contralateral to target finger stimulation.

The experimental group was comprised of 25 normal adult volunteers. All subjects were screened for disorders of skin sensation, bone fractures, head trauma, and brain disease. It is the hypothesis of this report that these obvious somatosensory EPs are accompanied by systematic changes in regional cerebral blood flow. Using impedance plethysmography procedures (rheoencephalography), it is possible to calculate a blood flow index which is highly correlated with cerebral blood flow. The rheoencephalographic method used in this study follows the procedure of Jacouy et al (1978).

Within the context of the identical selective attention paradigm, rheoencephalographic data were obtained to test the hypothesis that mental activity which elicits robust cerebral electrical potentials is accompanied by measurable cerebral blood flow changes.


Text structures can be studied through the notions of theme (information on which the text is built up) and rhyme (what the text informs about its theme). This contextual and topological perspective is covered by the pair Žorčič and comment at the level of sentences. Linguistic stimuli have already been demonstrated to evoke EEG responses correlated to functions and properties of natural languages. Our purpose here is to report the existence of correlations between evoked EEG responses and thematic structures of texts, the dynamics of such correlations, and their linguistic relevance. We presented a previously recorded text to the subjects while their EEG was recorded and immediately after, asked them to reproduce it in written and then to point to its theme. The whole procedure was repeated twice within an interval of 30 minutes. Results can be thus summarized: i) two context dependent strategies were used by the subjects during text reproduction; a divergent one, in which the theme was given in the first sentences of the reproduction, and the rhyme followed, and another, convergent, in which a set of arguments was given first, and then a theme followed; ii) the thematic structure defining elements evoked a characteristic electrical activity pattern; iii) the EEG activity reflected accurately by the syntactic or semantic performance of the subjects; iv) in the subject's convergent approach to the text, only comments evoked marked patterns in the EEG, while the most divergent strategy was used both topics and comments evoked responses. The above data seem to indicate: a) the existence of response evoked by the syntactic or semantic pattern and topological structures of natural languages; b) that the relationship between such structures depends on a context dependency. Exemplary EEG and thematic data can be obtained from the EEG by means of an adequate methodology which takes into account pertinent theoretical parameters.

EFFECT OF CHRONIC HALOTHANE EXPOSURE ON SENSORY EVOKED POTENTIALS IN FREELY BEHAVING RATS. G. M. Fuller, B. M. Rigonu, R. C. Wiggins and N. Daphy. Dept. of Neurobiol. & Anat. and Anesthes., Univ. of Texas Med. Sch., Houston, TX 77025.

Chronic halothane exposure has been implicated in pathologic changes in several peripheral organs. The present study was initiated to determine if chronic halothane exposure has any effect on bioelectrical activity recorded simultaneously from two different deep brain nuclei. Sensory evoked field potentials were employed as a measuring tool to assess halothane induced alterations in bioelectrical activity. The target nuclei in the present study were the mesencephalic central gray and the nucleus parafascicularis, two deep structure mediators of analgesia and anesthesia. Permanent semimicroelectrodes (60u diameter) were implanted stereotaxically under pentobarbital anesthesia several days prior to experimentation. The averaged acoustical evoked response (AER) following 32 repetitive click stimuli was recorded from both regions in halothane naive freely behaving rats on day 1. The rats were subsequently exposed to 0.25% halothane delivered through a vaporizer (Fluotec 3) with compressed air for 3 hrs per day, 5 days a week. AER recording was resumed at 28 and 56 days after the initial daily exposure of halothane. The AER consists of a positive (P1)-negative (N1)-positive (P3) wave. The last three components were consistently observed within and between animals and were therefore quantitated and compared to naive values. After 28 days of chronic exposure, no significant changes were observed in the recording obtained from the central gray as compared to the day 1 recording. In contrast to this, in the nucleus parafascicularis, the P2 and P3 components showed a statistically significant (p<0.01 and 0.05 respectively) increase in amplitude (paired t test). Following 56 days of halothane exposure, the P2 and P3 components of the central gray AER were statistically increased (p<0.05 and 0.005 respectively) over naive values. In the nucleus parafascicularis, the P2 and P3 components remained increased as at 28 days but with a more significant value for P3 (p<0.005). Thus although a general increase in AER amplitudes is seen in both regions examined, the time course and magnitude of change vary markedly. These data demonstrate a pronounced alteration of sensory evoked potentials following chronic halothane exposure. (Supported in part by USPHS grant no. NS-16355.)

Analysis of morphological and topographical relationships between scalp and intracranially recorded evoked potentials is fundamental to inferring the cerebral origins of scalp activity. One approach is to record from the two areas under conditions known to alter scalp potentials and to determine the effects of these conditions upon intracranial potentials. Scalp recorded somatic (SEP) and auditory (AEP) evoked potentials undergo dramatic changes during sleep. Therefore we are comparing scalp and intracranial recordings during waking and different stages of sleep. To date five patients have been studied; they had up to six multicontact depth probes chronically implanted bilaterally in frontal, central, and occipital regions as part of an evaluation for possible neurosurgery to relieve intractable epilepsy. Simultaneous recordings were made from 16 contacts selected from up to 108 available contacts on the probes. In all patients, the gross morphological changes from waking to sleep seen in scalp potentials were also observed intracranially. In four patients, long latency SEPs and AEPs had different scalp-depth relationships during waking whereas scalp-depth relationships were similar for the two modalities during sleep. Emphasizing the tentative nature of data from a few patients with abnormal brain, the observations are consistent with our previous suggestion (Goff et al., 1966) that long latency potentials occurring during waking and sleep reflect activity of different neural generators.

EFFECTS OF GENOTYPE, AGE, INTERSTIMULUS INTERVAL (ISI), AND BINARIAL STIMULATION ON COCHLEAR AND AUDITORY BRAINSTEM EVOKED RESPONSES (BSER's) IN THE LABORATORY HOUSE. Kenneth R. Henry, Dept. Psychol., University of California, Davis, Ca. 95616.

Several recent studies have shown that, as the ISI is decreased, the amplitudes and latencies of the various components of the BSER show differing recovery rates. These responses also differ in the human neonate, adult, and geriatric adult (Fujikawa and Weber, 1977). A binaural occlusive effect also occurs with PI IV in the cat (Huang and Buchwald, 1978) and Py in man (Blevad, 1975).

Mice of the C57BL/6 and CBA/J strains, from 20 to 380 days of age, were used for these studies. The former genotype expresses a progressive postpuberal hearing loss, while the latter maintains its hearing throughout its life (Henry, 1979).

As the ISI was decreased from 100 to 50 to 25 to 12.5 msec, latencies increased by 66, 141, 163, 188, and 356 usec for PI-Py, respectively, regardless of age and genotype. For the CBA genotype, decreasing the ISI reduced all amplitudes by approximately 45%. The C57BL/6 mouse also had this same 45% amplitude decline for PI, Py, and Py, but its PI III and Py responses only declined by 25% with decreasing ISI.

When monaural and binaural responses were compared, all ages and genotypes showed the same effect. For PI-V, binaural stimulation produced the same responses as did summing the responses obtained by monaurally stimulating the two ears. For PI-Py and Py, binaural stimulation reduced the amplitude by 25% and 60%, respectively.


Inferences could be made for the genesis of the underlying morphological differences which would have to go back to the early ontogenetic development of the brain when it still consists of a neural tube. The manner in which the ablateral holosphere becomes bilateral and develops into lobes as we know them would be at issue.

669 A MICROPROCESSOR SYSTEM FOR CORTICAL AND BRAINSTEM EVOKED POTENTIAL CORRELATES OF SEX AND SCHIZOPHRENIA. Duilio Giannitrapani, Veterans Administration Medical Center, Perry Point, MD 21902.

EEG sex differences, with particular reference to power, phase angle and coherence spectra, were compared to those found in schizophrenia. Eighteen males and 16 females, 11 to 13 year-old right preferents, were selected. Subjects were administered an EEG during which spectra were obtained from 16 brain areas between 2 and 34 Hz in 16 frequency bands, each 2 Hz wide.

Power spectra show overall greater power in the males except in the left occipital area in the 2 - 10 frequency range where the opposite was true for the females. The areas in which the females show greater power slowly broaden with increasing frequency. The broadest distributions occur in the 19, 21, 29 and 33 frequency bands, possibly indicating a broader distribution of the 2nd and 3rd harmonic of dominant activity in females, also found in schizophrenics by this investigator.

In the phase angle analysis in the 13 Hz activity, males show predominant significant anterior leading while females show significant posterior leading. A similar pattern has already been shown by this investigator for schizophrenics and normal adults respectively.

Coherence in general is higher in females in anterior areas and in males in posterior areas bilaterally. An exception is the 29 and 33 Hz activity, showing higher coherence in females in all comparisons, also found by this investigator in schizophrenics.

The 13 Hz phase angle finding and the 29 Hz power and coherence spectral finding are in contradiction to regard to the hypothesized sex-relatedness of schizophrenia. The 13 Hz finding is consistent with greater vulnerability toward schizophrenia in males while the 29 Hz data for the females correlates with higher power and coherence found by this investigator among schizophrenics. An attempt is made to coordinate the data from the three parameters studied and relate them to the known unequal distribution of psychological incidence among the sexes.

The data points to a physiological substratum of schizophrenia. Inferences could be made for the genesis of the underlying morphological differences which would have to go back to the early ontogenetic development of the brain when it still consists of a neural tube. The manner in which the ablateral holosphere becomes bilateral and develops into lobes as we know them would be at issue.

671 A MICROPROCESSOR SYSTEM FOR CORTICAL AND BRAINSTEM EVOKED RESPONSES, Steve H. Graham and M. E. Miner. Division of Neurosurgery, University of Texas Medical School, Houston, Texas 77030.

Recent advances in large scale integration have brought computer technology within the budget of almost every electrophysiological laboratory. The microprocessor, due to its programmability, can be used to control external devices such as stimulators, allowing for correction if necessary. The waveforms and other data peaks determined by the computer are displayed on an oscilloscope, and on the BASIC program that controls the sampling interval, number of points and number of repetitions to be averaged. Maxima and minima of the evoked potentials may be identified and their latencies from the stimulus computed. The peaks determined by the computer are displayed on the oscilloscope; manual review of the waveform and positioning of a cursor allows for correction if necessary. The waveforms and other data are stored on the floppy disk for later review and analysis. The A/D converter utilized has 8 bit resolution; therefore, one must compromise between maximal resolution and the possibility of the A/D converter being saturated. We have partially resolved this dilemma by not including any data in the sum if any of the channels contain a saturated data point. The parallel port is used to control external devices such as stimulators, allowing the microprocessor to supercede delay timers. Stimuli intervals may be randomized to prevent habituation.
673 May provide an important clinical tool for the assessment of.

675 Pilot study conducted with ICP monitoring suggest that the VEP measured by differential recording from surface electrodes at.

676 Previous studies have suggested that increases in positive and negative going wave component was identified by subtractive analysis in a microprocessor which assigned latency values to each peak. Latency values for eight component peaks of the VEP up to 256 msec were then correlated at 21 different increments of measurement.

677 The results suggest that the peripheral mechanisms were relatively preserved. These findings agree with the hypothesis that increases in ICP would cause consistent shifts in latency of waves constituting the visual evoked potential (VEP).

678 These results indicate that, with mixed nerve stimulation, the fast, early portions of the SSEP are probably due to muscle afferents, while the later, slower components result from cutaneous fibers.

679 In the monkey, the nerves were surgically exposed and stimulated with a stimulus intensity that produced an earlier response with an altered waveform. In contrast, no threshold effects were observed with stimulation of the sural and deep peroneal nerves. Stimulation of the tibial nerve at levels below motor threshold produced a response at approximately the same latency as that for the sural and deep peroneal nerves.

680 The relative contributions of cutaneous and muscle afferents to the somatosensory evoked potential is well established. In this study, the SSEP elicited by mixed nerves (the common peroneal at the fibular head, and the posterior tibial at the ankle) were compared to the superficial peroneal nerves (the deep peroneal nerve distal to the bifurcation of the lateral and medial branches, and the sural nerve at the ankle) were compared in man and in the stump-tail macaque monkey.

681 In the human, the response to stimulation of common peroneal (mixed) nerve at levels below the motor threshold was almost identical to that obtained with supramaximal stimulation of the sural and deep peroneal (cutaneous) nerves. With increased stimulus intensity, the common peroneal response appeared at a shorter latency and, in some cases, with an altered waveform. In contrast, no threshold effects were observed with stimulation of the sural and deep peroneal nerves. Stimulation of the tibial nerve at levels below motor threshold produced a response at approximately the same latency as that for the sural and deep peroneal nerves. Stimulation of the deep peroneal nerve produced an earlier response with an altered waveform.

682 These results indicate that, with mixed nerve stimulation, the fast, early portions of the SSEP are probably due to muscle afferents, while the later, slower components result from cutaneous fibers.

Short latency evoked potentials were first recorded from the intact skull of the cat, using averaging techniques. Subsequently, an amphetamine free series of far field deflections were recorded following click stimuli in the intact human. Evoked potentials have proved clinically useful in patients with lesions of the brain stem.

Methods: A laminar analysis of the auditory evoked potential waves was made in 19 barbiturate-anesthetized cats. Fine stainless steel wire electrodes were placed stereotaxically at a calculated oblique angle into the superior olivary nucleus (SO), the lateral lemniscus (LL), and the inferior colliculus (IC). Conventional responses were recorded from an electrode at the vertex, all recordings using a reference in the pinna. Clicks were delivered through hollow ear bars. The recording parameters and averaging and read-out routines were the same as those used by other workers.

Results: A series of 4 to 6 positive waves were recorded from the vertex, identical to those reported by others. These ranged up to 6 or 7 ms. in latency, and had amplitudes in the 1 microvolt range.

The potentials recorded directly from the depth in the brain stem were of much greater amplitude, so that they could clearly be seen on the oscilloscope face even without averaging. At SO, and IC, biphasic waves were recorded. As the electrode was advanced gradually, the waves remained of uniform configuration over a length of 4 or 5 mm. Thus, the locus of maximal amplitude could easily be found. This was presumed to be the center of the neural activity.

Latency of the first of the series of waves of each evoked potential was shortest at SO. The first wave occurred progressively slightly later at LL and at IC. The amplitude of the waves was clearly greatest at SO, and successively somewhat lower at LL and at IC.

The precise latencies of the peaks of the waves recorded from the several brain stem electrodes did not all exactly correlate with each other, nor with the exact latencies of the peaks recorded from the vertex.

These results agree with the preliminary descriptions of Starr and Achor. It is clear that a simplistic "generator" theory of the origin of the vertex-recorded auditory evoked potential is untenable. A more complex analysis, analogous to that of the electrocardiogram recorded from the surface of the body, is needed.

Contributions to the SEP by the major ascending somatosensory pathways. Richard K. Simpson Jr., John G. Blackburn, Henry F. Martin and Sidney Katz. Department of Physiology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29403.

The contributions of various spinal cord pathways to the somatosensory evoked potential (SEP), was investigated in monkeys anesthetized with 70% N₂O and 30% O₂. SEPs were recorded in response to stimulation of exposed superficial peroneal nerves. Stimulus intensities were sufficient to excite either large diameter nerve fiber groups or all nerve fiber groups. While applying maximal stimulus intensities, the large diameter nerve fiber groups were blocked using a combination cooling and electrical polarization, (Blackburn, J.G. and Katz, S. 1976., J. Electrophysiol. Tech. 5: S1-7). The procedure allowed selective small diameter nerve fiber input to continue. SEPs were recorded in response to stimulating various peripheral nerve fiber groups following either bilateral dorsal or anterolateral transection, left or right hemisection, and central cord lesion or complete cord transection at levels T3-T4. The results indicate that only the dorsal column system, (including the spinocerebellar tract), contributes to components P1 and N1. Only the anterolateral column system contributes significantly to components P3 and N5. Both the dorsal and anterolateral column systems contribute to components P2 and N2. It appears that small diameter nerve fibers and the anterolateral column systems contribute significantly to the later wave components of the SEP. The observation that both major ascending spinal cord pathways contribute to various components of the SEP enhances the possible utility of the SEP as a method of evaluation and treatment of human spinal cord injury. (Supported by NINDS grant P-5P81-N5-11066).


This presentation reports the initial results of a rational, stepwise analysis of evoked potential (EP) measures conducted for the purpose of predicting clinical psychological characteristics by electrophysiological methods. Subjects were a diagnostically homogeneous, conservatively tested group of severe, chronic psychotic patients. Somatosensory (SEP), visual (VEP) and auditory (AE). EPs were recorded in one session, using pseudo-randomly presented left (LSEP) and right (RSEP) median nerve shocks, checkerboard pattern flashes and binaural auditory clicks. Recordings were made from one EDR and 14 scalp leads. Clinical criteria were as follows: (a) psychiatric diagnosis discharge diagnosis independently made by two senior psychiatrists; (b) symptom ratings—16 Brief Psychiatric Rating Scale (BPRS) items rated by a psychiatrist; (c) intelligence—Raven's Progressive Matrices.

Visual detection of EP peaks in key leads provided estimates of the latency of each of 11 or 12 consecutive peaks (depending on the type of stimulus). The estimates were used to automatically determine the amplitude of each peak in all scalp and depth records.

The first stage of analysis was to reduce the dimensionality of the data by extracting the factors involved in the spatial distribution of the vertex-recorded auditory evoked potential (AESEP). A balanced rotation and analysis of the amplitudes for a given peak at all leads usually resulted in the extraction of five factors, one of which often reflected EEG activity. The resulting factor scores for all peaks of a given type of EP for the patient were subjected to hierarchical cluster analysis. Using RSEP factor scores as an example, six distinct clusters of patients were identified, and the results to far obtained indicate that two of these clusters contained a large weighting of depressive patients. Other RSEP clusters were significantly related to the intelligence measure and to ratings of anxiety.

The encouraging results justify further research aimed at classifying psychiatric patients by means of EPs to identify clinically meaningful subgroups that transcend conventional diagnostic categories.

Support (in part) by USPHS Grant MH 12507.

Auditory brain stem evoked potentials in autism. Barry F. Skofl, Allen P. Mirsky, Laboratory of Neuropsychology, Boston University Medical Center, Boston, MA 02118.

Current theories of childhood autism suggest that the dysfunction is an organic one, primarily affecting perceptual and language mechanisms. Furthermore, it has been suggested that pathology involving the brain stem could explain many of the symptoms commonly seen in autistic children. The auditory brain stem evoked potential (ABSEP) is a simple, non-invasive method of assessing the integrity of the brain stem areas through which the auditory system courses.

In the present study, ABSEPs were acquired in a group of 20 children who had been diagnosed as autistic, or who exhibited autistic-like behaviors. Click stimuli (100 Hz, 60 dB HL) were presented monaurally to each ear, while the filtered (300-3 Khz) EEG was recorded. Signal averaging was done off-line with a fast rejection technique.

Data from 3 children could not be evaluated. Of the remaining 17, 6 (35%) had abnormal ABSEPs when compared to a group of normal control subjects. One child had a prolonged latency in the children apparently had no response when the other ear was stimulated. The remaining child had early waves I through III, and no detectable wave V (midbrain/inferior collicullus).

It appears that some form of brain stem pathology exists at least some children that are diagnosed as autistic or exhibit autistic symptoms. The next step in elucidating the etiology of the syndrome is to determine whether there exists a correlation between brain stem pathology as shown by the ABSEP and specific behaviors or symptoms. The ABSEP may be eventually a simple and painless method of screening for early detection of childhood autism.

Supported in part by Biomedical Research Support Funds of Boston University Hospital and grant NS-112201 from the Public Health Service.
Cortical representation of the body surface is of intrinsic interest to neurologists. Correlation of easily replicable, recognizable, natural sensations with cerebral electrophysiology is useful for understanding the functional significance of event related brain potentials (ERBP). Marginal mucocutaneous skin of anogenital regions has these advantages over other peripheral sites for recording somatosensory evoked potentials (SEP): (1) Transcutaneous electrical mini-pulses (0.03-0.08 ms), suitably entrained, elicit only tactile, superficial, and strictly spatially discrete sensations, without shock overtones, for a wide range of qualities and magnitudes; (2) SEP so elicited are amplitude-focal at vertex, without polarity reversal laterally or evidence of electro-ocular interference; (3) risk of local muscular reflex is low (contrasted to median and other nerve-trunk stimulation); and (4) stimulation and recording sites are distant (compared to lip and orodental sites, where sensation also tends to radiate).

For formal informed Consent reasons, this intensive study was confined to two volunteers: an experienced male who self-paced his stimulus time-window, and a relatively naive female who received conventional, regularly repetitive stimulation programmed by the experimenter. Unequivocal results: (1) Anogenital SEP has later cortical processing times (peak latencies, 30-250 ms) than SEP for comparable stimulation, electrical and mechanical, eliciting comparable sensory magnitudes but different qualities, on hand and foot; also higher mean frequency in the intermediate time segment (50-175 ms) than is recordable for stimulation at other extremities; (2) its dipole origin seems not to be oriented normal to the gross medial surfaces of the hemispheres, as expected from the classical neuroanatomy. It is hoped the above advantages will outweigh possible psychosocial disadvantages. This form of input might be a more sensitive probe than median nerve shock for diagnostic SEP recording in prospective surgical cases.

This work was supported by Research Section of PERT of DMHE & MR, State of Georgia, USA.


It is often the case that multiple electrode derivations are used to record and identify specific peaks of BAEPs. This usually entails recording from two vertex-mastoid pairs, one from each mastoid side and a common vertex as "G1". In cat both derivations, that is, the generator for wave 1, may not be the same region which generates wave-2, even though they are recorded simultaneously. Ordinarily, the amplitudes and latencies are commonly seen between the two records used to record and identify specific peaks of BAEPs. This is easily replicable, recognizable, natural sensations with cerebral electrophysiology is useful for understanding the functional significance of event related brain potentials (ERBP). Marginal mucocutaneous skin of anogenital regions has these advantages over other peripheral sites for recording somatosensory evoked potentials (SEP): (1) Transcutaneous electrical mini-pulses (0.03-0.08 ms), suitably entrained, elicit only tactile, superficial, and strictly spatially discrete sensations, without shock overtones, for a wide range of qualities and magnitudes; (2) SEP so elicited are amplitude-focal at vertex, without polarity reversal laterally or evidence of electro-ocular interference; (3) risk of local muscular reflex is low (contrasted to median and other nerve-trunk stimulation); and (4) stimulation and recording sites are distant (compared to lip and orodental sites, where sensation also tends to radiate).

Binaural interaction was examined by recording auditory brainstem responses to clicks from scalp electrodes (vertex-neck) in human subjects. Deviations of the binaural response from the sum of the monaural potentials were observed at the time of occurrence of waves V-VII. Both amplitude and latency of the interactions were dependent on click polarity: rarefaction clicks produced interactions of larger magnitude relative to the monaural sums (38.2±9.32) and longer latency (6.26±0.65 ms.) than did condensation clicks (25.4±4.41; 5.34±0.63 ms.). These latency differences cannot be accounted for by any concurrent latency shifts in the monaural or binaural evoked potentials. The relationship of binaural interaction to stimulus intensity, interaural time and intensity differences, and audiograms were examined.

Supported by NIH grant NS11876.
PSEUDORANDOM BINARY SEQUENCE (PRBS) STIMULATION FOR VISUALLY EVOKED POTENTIALS (VEP). Weldon W. Wright, Richard Srebro and Barry A. Sokol. Department of Ophthalmology, Southwestern University of Texas, Dallas, Texas 75235. PRBS stimulation permits a rapid measurement of the power spectrum of the VEP with an input that approximates bandlimited white noise but which is amenable to signal averaging techniques. We have applied the method to both luminance modulation of a 30° homogeneous red field and to pattern reversal of sinusoidal and square wave gratings. Power spectra were obtained in less than 2 minutes of recording time. These spectra are similar to those obtained using an array of single sine wave frequencies each presented separately but the PRBS technique requires much less recording time and has the additional advantage that separate epochs are not required for each frequency tested. The PRBS consists of a repeating sequence of N equally likely binary states. It simulates a true coin toss experiment in that the number of runs of consecutive identical states of length \( N \) is proportional to \( (1/2)^n \). State changes are allowed only at equally spaced time intervals, \( \Delta t \), and the sequence has a periodicity of \( N \Delta t \). In the frequency domain, the PRBS is represented by a set of discrete equally spaced pulsed of constant amplitude in the frequency band from \( 1/N\Delta t \) to \( 1/3 \Delta t \). The PRBS can be conveniently generated using a shift register with appropriate feedback. An n stage shift register generates a PRBS whose length is \( N = 2^n - 1 \). Selecting n and \( \Delta t \), permits adjustment of the frequency resolution and bandpass appropriate to the system being studied. We have generated PRBS stimuli from both an inexpensive hardware shift register and by simulation using a small computer. For luminance modulation the binary states were made to coincide with 2 adjustable luminance levels so that average luminance and % modulation were precisely and independently adjustable. For pattern reversal experiments the position of the gratings on an oscilloscope was shifted by \( 1/2 \) cycle left or right in accordance with the binary state of the PRBS. The VEPs were recorded both by using a conventional signal averager and were later digitized, or were averaged directly by the lab computer. A Blackman-Harris spectral window was applied to the VEP before taking the Fourier transform. PRBS stimulation permits a rapid measurement of the power spectrum of the VEP with an input that approximates bandlimited white noise but which is amenable to signal averaging techniques. We have applied the method to both luminance modulation of a 30° homogeneous red field and to pattern reversal of sinusoidal and square wave gratings. Power spectra were obtained in less than 2 minutes of recording time. These spectra are similar to those obtained using an array of single sine wave frequencies each presented separately but the PRBS technique requires much less recording time and has the additional advantage that separate epochs are not required for each frequency tested. The PRBS consists of a repeating sequence of N equally likely binary states. It simulates a true coin toss experiment in that the number of runs of consecutive identical states of length \( N \) is proportional to \( (1/2)^n \). State changes are allowed only at equally spaced time intervals, \( \Delta t \), and the sequence has a periodicity of \( N \Delta t \). In the frequency domain, the PRBS is represented by a set of discrete equally spaced pulsed of constant amplitude in the frequency band from \( 1/N\Delta t \) to \( 1/3 \Delta t \). The PRBS can be conveniently generated using a shift register with appropriate feedback. An n stage shift register generates a PRBS whose length is \( N = 2^n - 1 \). Selecting n and \( \Delta t \), permits adjustment of the frequency resolution and bandpass appropriate to the system being studied. We have generated PRBS stimuli from both an inexpensive hardware shift register and by simulation using a small computer. For luminance modulation the binary states were made to coincide with 2 adjustable luminance levels so that average luminance and % modulation were precisely and independently adjustable. For pattern reversal experiments the position of the gratings on an oscilloscope was shifted by \( 1/2 \) cycle left or right in accordance with the binary state of the PRBS. The VEPs were recorded both by using a conventional signal averager and were later digitized, or were averaged directly by the lab computer. A Blackman-Harris spectral window was applied to the VEP before taking the Fourier transform.

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FEEDING AND DRINKING
THE EFFECT OF RESTRICTED FOOD AVAILABILITY SCHEDULES ON CIRCADIAN RHYTHMS. Norman T. Adler and Rodney J. Pelchat. Department of Psychology, Univ. of Delaware.

Much recent work has suggested that food restriction schedules (FR) can entrain or synchronize circadian rhythms. We have performed a series of experiments to compare the entraining effects of food availability cycles to the known effects of light cycles on two rhythms - wheel-running activity and food-getting operant. Residual effects of entrainment by food are clearly not fit the classic effects of entraining agents.

First, although there is an anticipatory wheel-running response to FR which can persist for two days of total food deprivation, FR does not entrain free-running activity rhythms of either blinded animals or animals in constant light. The neural structures supporting the latter rhythms are intact and food deprivation is insufficient to entrain these rhythms.

Second, the effect of a shift from FR to ad libitum food while concurrently on a light-dark cycle is different for the feeding and activity rhythms. If food availability were entraining an oscillator, then residual effects of this entrainment might be expected at the old feeding time. Residual effects for the operant feeding response but not for wheel-running were observed.

Third, extra access to food either as an extension of the normal feeding time or as a separate feeding schedule to accommodate restricted food availability seems to affect the expression of circadian food-related rhythms. In both groups, residual effects of both wheel-running and the food-getting operant were observed. The results suggest that the food-availability cycles are not acting as classic Zeitgeber and that mechanisms other than entrainment of an oscillator are at work.


Withdrawal of ovarian steroids by OVX results in a temporary increase in food intake with a concomitant increase in BW. Food intake generally returns to control levels when BW reaches a new, higher level. The primary effect of hyperphagia may be to alter the level at which body fat is regulated, i.e., the 'body fat set point.' Recent data shows that rats with DML have experienced a lowering of the 'set point' for BW but not for body fat (Bellinger, et al. Neuroscience, in press). The present study was designed to investigate whether OVX would be able to raise the body fat 'set point' in DML rats. Rats received DML at the age of 28 days, sham-operated rats served as controls. At the age of 84 days some DML and control animals were OVX while others were sham-OVX. BW, food intake and obesity index were monitored and the experiment terminated when the rats were 140 days old. Both OVX and sham-OVX caused precipitous drops in BW that lasted only three days; however, the BW drop was greater in the sham-lesioned than in the DML rats. Subsequently, the patterns of BW increase were identical in the DML-OVX and sham-lesioned-OVX rats, the curve of the former being more shifted to a lower absolute BW. The data allows the inference that OVX causes the same degree of BW gain in DML and sham-DML rats and thus cannot override the BW-lowering effect of DML. Deposition of body fat as evidenced by the increase in the fat index was same in DML-OVX rats than in any of the other three groups of rats. The data suggests that DML, although lowering BW on an absolute basis, predisposes the rats to the lipogenic consequences of obesity. Similar to BW, the post-operative drop in food intake was smaller in DML rats than in sham-lesioned rats when OVX was performed. Subsequently, for 27 days, the food intake of DML-OVX rats ate more than DML-sham-OVX animals, but thereafter food intake normalized. By contrast, sham-lesioned rats responded to OVX by increasing their food intake for 27 days, whereas when the neural structures involved in regulating the body fat 'set point' seems intact and more responsive to manipulation, i.e. OVX. Finally, as reported by others OVX may lead to obesity by more than solely affecting food intake as illustrated in the DML-OVX group.

INSULIN-INDUCED ELEVATION OF HYPOTHALAMIC NE TURNOVER PERSISTS AFTER GLUCORESTORATION UNLESS FEEDING OCCURS. Steven I. Beilin. Veterans Administration Medical Center and Depts. of Surgery and Pathology, SUNY at Buffalo, 14215 and Dept. of Physiology, Baylor Coll. Dent., Dallas TX 75246.

Sprague-Dawley rats received bilateral electrolytic lesions in the DML at the age of 49 days. Sham-operated rats served as controls (CON). The rats were originally used in various feeding studies. At the age of 183 days they were subjected to TP for 30 minutes per day and a control period of 30 minutes. The feeding rhythms, complete in all nutrients and accessory food factors, with one macronutrient higher than the other two, were available ad libitum during the 30 minutes control period. When insulin-treated rats, the insulin-induced increase in hypothalamic NE turnover was abolished. These results suggest that insulin-induced increases in NE turnover in the hypothalamus may be specifically related to food intake and not to other signs of glucoprivation.

TAIL PINCH (TP)-INDUCED HYPERPHAGIA AND MACRONUTRIENT PREFERENCE IN YOUNG-NATURALLY MALE RATS MADE HYPERPHAGIC BY LESIONS IN THE DORSOMEDIAL HYPOTHALAMIC NUCLEI (DML). Lee L. Bernardi and Larry L. Bellinger. Veterans Administration Medical Center and Depts. of Surgery and Pathology, SUNY at Buffalo, 14215 and Dept. of Physiology, Baylor Coll. Dent., Dallas TX 75246.

Sprague-Dawley rats received bilateral electrolytic lesions in the DML at the age of 49 days. Sham-operated rats served as controls (CON). The rats were originally used in various feeding studies. At the age of 183 days they were subjected to TP for 30 minutes per day and a control period of 30 minutes. The feeding rhythms, complete in all nutrients and accessory food factors, with one macronutrient higher than the other two, were available ad libitum during the 30 minutes control period. They were a high-carbohydrate (HCD), high-fat (HFD) and a high-protein (HPD) diet. In their home cages, the rats received Charles River Rat Mouse Harvester Formula ad libitum. Rats with DML lesions (DML rats) ate significantly more than their sham-operated controls from each of the three diets during TP sessions, but particularly from the HCD and the HFD diets. Nevertheless, in terms of per cent intake, the DML rats and the CON rats ate similar amounts of food from each of the three diets. In their home cages, the DML rats showed the previously reported profound hypophagia. Indeed, food intake in the home cages showed over time a trend to decrease in the DML rats and a trend to increase in the CON. The data show that the mature DML rat, as its weanling counterpart, is grossly hypophagic in its home cage but shows stimulus-bound hyperphagia when subjected to TP. Similarly the mature DML rat shows preference for a HCD and HFD diet, as does the weanling DML rat. The data also show certain similarities to the stimulus-bound hyperphagia in rats with lateral hypothalamic lesions (LHA rats) which have been shown to be capable of increased food intake during the critical post-operative time until recovery, i.e. spontaneous food intake, supervenes. However, high stage hypophagia and adipisia. The possibility is entertained that DML cause a partial disruption of some inhibitory tracts, possibly part of the noradrenergic bundle, thus disinhibiting feeding on TP the dopaminergic (DA) system in vivo more actively in DML than in CON rats.

Supported by NSF Grant PCM76-84381.

Supported by NSF Grant PCM76-84381.

It has been previously shown that rats less than 14 days of age suck in 2 different patterns: rhythmically and in an arrhythmic fashion. This study was designed to determine whether arrhythmic sucking is elicited by depolarizing agents. Pups of 10- to 14-day-old, short (4-6 hr) and long (20-24 hr) deprived pups with methysergide (20 mg/kg) or the saline vehicle (10 ml/kg). Twenty min later their sucking for lhr using an EMG recording technique designed to measure the frequency, duration, and intensity of dry sucking (sucking from an unanesthetized dam who provides no milk).

THE EFFECTS OF HEPATIC VAGOTOMY ON SALT INTAKE AND BODY WEIGHT

Hepatic vagotomized (HV) rats ingested less NaCl solution (0.15% NaCl) than control rats (SC). However, in both groups the NaCl intake was less than the water intake. The differences in average body weight agree with the results reported in other laboratories (Adachi, Niijima and Jacobs, 1976). The appetite appears with a latency of several hours after initiation of the AIH infusion and persists after its termination. When 0.9% NaCl is substituted for 3% NaCl in the diet of rats, both 0.9% NaCl and water occur during cICV of AIH (6gc/hr/lhr), however, unlike the 3% NaCl and water intakes, more 0.9% than water is drunk (17.4±3.3 water and 14.5±1.7 NaCl). In addition, rats given cICV of AIH during the last 4 days also drank more NaCl during the first 4 days than AIH-free diet throughout the entire experiment. However, 24 hr after the last 24 hr of drinking of NaCl, rats given AIH-free diet for 5-13 days with or without cICV of AIH also drank significantly more, but this occurrence occurred only in those receiving cICV during Na+ deprivation. Thus, 1) it is not necessary that the animals drink salt solutions during AIH infusion in order for the hormone to evoke salt appetite, and 2) salt ingestion is either in solution or in solid food during chronically elevated AIH, produced either by cICV or by Na+ deprivation, appears to be necessary in order for the excess salt intake to persist. Supported by MH07172-01, MH07753-01, NINCDC03469.

THE EFFECTS OF HEPATIC VAGOTOMY ON SALT INTAKE AND BODY WEIGHT

FEEDING AND DRINKING

SCMN treat antimuscarinic drugs. Secretory effects of parasympathetic stimulation, probably to compensate for the absence of saliva. The differences in average body weight agree with the results reported in other laboratories (Adachi, Niijima and Jacobs, 1976). The appetite appears with a latency of several hours after initiation of the AIH infusion and persists after its termination. When 0.9% NaCl is substituted for 3% NaCl in the diet of rats, both 0.9% NaCl and water occur during cICV of AIH (6gc/hr/lhr), however, unlike the 3% NaCl and water intakes, more 0.9% than water is drunk (17.4±3.3 water and 14.5±1.7 NaCl). In addition, rats given cICV of AIH during the last 4 days also drank more NaCl during the first 4 days than AIH-free diet throughout the entire experiment. However, 24 hr after the last 24 hr of drinking of NaCl, rats given AIH-free diet for 5-13 days with or without cICV of AIH also drank significantly more, but this occurrence occurred only in those receiving cICV during Na+ deprivation. Thus, 1) it is not necessary that the animals drink salt solutions during AIH infusion in order for the hormone to evoke salt appetite, and 2) salt ingestion is either in solution or in solid food during chronically elevated AIH, produced either by cICV or by Na+ deprivation, appears to be necessary in order for the excess salt intake to persist. Supported by MH07172-01, MH07753-01, NINCDC03469.
Therefore, the relative overeating and obesity which can develop on diets high in fat is an interesting model of apparent break­
centration. This latter factor is rarely considered despite abun­
rats were housed separately in a temperature (22°C±1°) and light
was either low or equal to that found in standard maintenance
diets. Adult (250-280g) male ( n=30) and female ( n=30) Wistar
ed normal). Male rats showed slight but significant linear growth
s continued linear growth of adult male vs female rats confounds as­
30-310 is considered normal). Clear sex differences in magnitude and
that such caloric enhancement simultaneously lowers protein con­
Experiment addressed this issue by comparing the development of obesity in high-fat and control diets in which the concentration of energy in the form of protein was either low or equal to that found in standard maintenance
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Sext Differences in Rat Obesity Produced by High-Fat Diets. D.V.
the area postrema (AP) is a circumventricular organ located
the caudal fourth ventricle. This structure, which is a known
chemoreceptor for certain drugs and toxins, has been shown to be
involved in the control of body weight and food intake. Mice geneti­
cally altered to lack the AP have a higher body weight and adiposity
relation between weight loss and food intake during the 30-day infu­
These results are interpreted as indicating that a variety of
substances involved in carbohydrate and lipid metabolism are also
involved in the control of body weight and food intake. It is likely
that the AP is an area through which control of food behavior can
We have made lesions of the AP in adult male rats. After
recovery from surgery, AP lesioned rats consumed normal amounts of
lab chow and had only slightly decreased caloric intake. This is
expected since AP lesioned rats increased their food intake by
amounts which were statistically indistinguishable from sham
operated rats and unoperated controls. However, when non-
operated AP lesioned rats were permitted to consume a highly
palatable liquid food (vanilla instant breakfast) during a
thirty minute test, they consistently ate more than double the
amount consumed by sham operated or control rats. For example,
in a typical experiment involving 10 lesioned rats and a pooled
sample of 5 sham operated and 5 control rats, the AP lesioned
rats ate 27.5 ± 2.5 mls whereas shams and controls averaged only
12.1 ± 1.0 mls. This increased intake by AP lesioned rats
appeared shortly after surgery and persisted for at least 6 months.
A second finding of note was that the sham operated control
food intake was not significantly different from that of the AP
lesioned rats. Therefore, we tested the possibility that AP lesion-
induced overeating resulted from reduced sensitivity of the
chemoreceptor to the satiety effects of cholecystokinin (CCK).
Intraperitoneally injected CCK octapeptide (20 or 40 lgy dog units/kg)
produced similar percentage decreases in food intake by both
lesioned and control rats. Our data suggest that the AP is involved in
termination of feeding induced by highly palatable liquid foods. The
effect of the AP on caloric intake by lesioned animals is therefore
attributed to decreased sensitivity to CCK. Therefore, disruption of
the AP may impair satiety by interfering with detection of, or response to, other satiety sig­
The large electrolytic lesions of the ventromedial hypothalamus (VMH) which produce hyperphagia and obesity in rat typically damage many nuclei and fibers and thus are not useful for the localization of neural substrates mediating satiety. More discrete lesions have shown that the critical area which must be destroyed lies rostral to the ventromedial nucleus (Gold et al, 1977). Using asymmetrical knife cuts Gold et al. (1977) revealed the coronal plane of the paraventricular nucleus (PVN) as the rostralmost terminus of the satiety neurocircuit. The PVN is the most sensitive brain site for norepinephrine-induced eating (Lesbovit, 1978). However, knife cuts of the tractus filiformis, a major noradrenergic tract to the PVN, paradoxically produce a transient reduction in food intake, despite a significant depletion of VMH norepinephrine (Crowley et al. 1978). In the present study we report that discrete lesions of the PVN produce hyperphagia and rapid weight gain in the rat without the additional deficits traditionally associated with VMH lesions. We therefore suggest a specific role for the PVN in satiety.

Bilateral adrenal lesions were made in 40 female rats using a platinum electrode (1 mm x 10 sec.). The lesions were aimed in a grid pattern centering on the PVN.

The greatest weight gains (7 g/d) were obtained when the PVN was completely and selectively destroyed. This rate is comparable to that of sham-operated controls. It is clear that the lesions significantly increased food intake. These findings are consistent with those of other investigators (Leibowitz, 1978). However, knife cuts of the tractus filiformis, a major noradrenergic tract to the PVN, paradoxically produce a rapid reduction in food intake, despite a significant depletion of VMH norepinephrine (Crowley et al. 1978). In the present study we report that discrete lesions of the PVN produce hyperphagia and rapid weight gain in the rat without the additional deficits traditionally associated with VMH lesions. We therefore suggest a specific role for the PVN in satiety.

The genetically obese Zucker rat represents a possible animal model for the experimental analysis of human obesity. The obesity results primarily from hyperphagia. Although food intake of the fatty rat (fa/fa) and its lean littermates has been studied with a variety of drug and dietary manipulations, no reports of eating following chemical stimulation of hypothalamic areas have been made. The literature concerning the neurotransmitter mediation of eating has typically focused upon noradrenergic systems as principle mediators of the response. Recent research has indicated that cholinergic stimulation of perifornical hypothalamic areas (PFH) also elicits dose-dependent eating in satiated rats (Chance et al., Physiol. Psych. 5: 440, 1977). In this experiment, we examined eating and drinking following cholinergic and noradrenergic stimulation of the PFH in 6 fatty and 6 lean littermate Zucker rats. The rats were anesthetized (chloral hydrate) and cannulae were implanted into the PFH. After at least 1 week to recover from surgery, the rats were placed on ground chow and water ad lib. An additional 7 day period of adaption to the new diet was allowed before the initial injections. Food and water intake was determined in satiated rats 1 hr following the initial control injection of normal saline (1 µl) as well as 1 hr following subsequent administration of carbachol (CAB; 4 µmol) or norpinephrine (NE; 24.0 nmol) with intake being expressed as mean different between these two periods. In the initial experiment CAB was administered daily for 5 consecutive days. Following a 2 day drug-free period, NE was administered for 3 consecutive days. Although there was no difference between groups, NE elicited significantly greater eating with a peak value of 6.2 g to 3.1 g by day 3. Following the injection of CAB, significant drinking was observed in both groups (overall mean = 6.2 g) with no differences apparent. Food intake following CAB was dramatically different between the two groups, with the lean littermates consistently showing significant eating across the 5 days (overall mean = 9.9 g). The fatty rats ate more during the control period than following CAB, on the first 2 days, however, this pattern changed by day 4 when there was no difference between the obese and lean rats. These data suggest that the adrenergic systems may be similar in fasted and normal rats. Although the physiological significance of eating elicited by cholinergic stimulation is at present unknown, genetically obese rats appear to exhibit an initial refractoriness to this stimulus. Supported by NSF Grant NS77-19302, NIH Grant 5 RO1 NS14344 and NIAAA Grant 5 RO1 AA01157.


In Experiment 1, meal patterns of intact female rabbits were measured through 65 to 67 h after an overnight food deprivation. Changes in food intake across the cycle were reflected differentially in other variables, depending upon the specific time of the cycle at which the variables were tested. Food intake occurring during the hours immediately following light offset was attributed to increased meal frequency and feeding rate, while the hours following the latter portion of darkness was attributed to increased meal duration and meal size. Meal patterning was also nonhomogeneous within the various portions of the 48 h feeding period. The meal patterns of intact animals from one period to another would suggest that this source of variation has been problematic in developing an understanding of short-term regulation of food intake. Experiment 2 was conducted to study the disruption of feeding patterns following vagotomization. TCA-ade demonstrated the effects of the time of the light-dark cycle when feeding patterns of vagotomized rabbits were different from those of intact rabbits and, further, delineated the relevant variables that were altered by vagotomization. During the first portion of the dark cycle, vagotomized rabbits were distinguished from intact rabbits by slower feeding rate and decreased total food intake. Interestingly, the meal patterning of vagotomized rabbits was not only significantly different from that of intact animals during the initial portion of the dark cycle but, furthermore, bore a resemblance to the feeding patterns of both intact and vagotomized animals during the latter portion of the dark cycle. During the light feeding light offset, vagotomized animals were distinguished from intact animals by decreased feeding frequency, increased meal duration, and increased satiety ratio. During the period immediately following either light onset or light offset, the feeding patterns of vagotomized animals could not be distinguished from those of intact animals. Supported by UCLA Research Grant (P30), M016665 (P30), MHS1010 (JAN), MHS1259 (ARNY), and N57687 (DH).


Rats intrahypothalamically injected with dopamine (DA) while 22 h food deprived and measured on consumption for 1 h immedi­ately thereafter failed to show a facilitation of food intake at baseline saline controls not only on that day but on similarly conducted saline injection tests administered thereafter every 6 days. This attenuation of food intake was observed in rats injected with DA while satiated and then tested. Not only these rats show facilitated intake if they were retested on their subsequent saline injection tests while 22 h food deprived. These data imply that DA-facilitated feeding is strongly linked to the state of the animal at the time of the DA injection and not to the state on subsequent saline tests. Given, but only given, that a reward mechanism is activated, as it is when a rat encounters food while hungry, does it appear that DA can enhance this activation. But once the enhancement occurs this facili­tation is reinforced and food intake is reflected on similar saline tests for some time thereafter.

In the preceding experiments the rats tested 22 hrs deprived neverless had free access to food between test dates; a further experiment placed severe constraints upon the feeding situation by routinely allowing the rats only 2 hrs daily access to food. After weight and intake stabilized, DA was intra­hypothalamically injected prior to the presentation of food. Food intake was not significantly increased. However, intake was not attenuated as has been reported by Hether, Ziegand and Stricker (J. Pharmacol. Exp. Ther. 99: 799, 1949) when 1.5 i.p. injections of DA agonists in a similar paradigm. These data suggest that under such temporal constraints the pressure to feed until satiated suppression was not activated and then a DA facilitated intake. A role of DA in modulating the rewarding consequences of food intake will be discussed further.


We have previously reported (Abstr.Soc.Neurosci.4:174,#529, 1978) that the gut-brain peptide bombesin (BBS) suppresses food intake in rats, and have suggested that BBS may play a role as a satiety signal when it is released by food during a meal. In the preceding experiments the rats tested at different times of the light-dark cycle at which the animals were tested in a situation in which other effective food-contingent satiety signals were minimized, we tested its satiety action in a sham feeding paradigm. (In this situation, rats eat almost continuously throughout a 60 min test--thus, no effective satiety signals operate.) Thirteen adult male Sprague Dawley rats were each surgically equipped with a chronic gastric stain cannula and then allowed a 22 h overnight food deprivation. Tap water was always available. BBS had a clear and rapid satiety action during sham feeding.

Percent Suppression of Food Intake 15 min after BBS Injection

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<th>Dose of BBS (µg/kg)</th>
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Throughout the 22 h overnight food deprivation period, BBS resulted in suppressed feeding. When BBS was presented during the 60 min test period, BBS resulted in suppressed feeding. This study was supported by U.S.P.H.S. Grant AM17240 and RSDA MH70874.
HYPOGLYCEMIC RESPONSE TO 2-DG. This unique finding suggests blood glucose levels than either the KC or control group. The LH group also had lower rather than to CNS intervention. The LH group also had lower that these knife cuts interfered with the sympathetic activation mobilization of free fatty acids (FFA) but do not affect the rise in blood glucose typically elicited by 2-deoxyglucose (2-DG) [Nishizawa & Gray, J. Clin. Invest. 61: 714, 1978]. Because VM lesions are associated with hypertension, obesity, and depression, the study examined the possibility that hypophagia-inducing lateral hypothalamic lesions (LH) lesions of mesopontomedullary knife cuts (placed lateral to the LH) differentially alter FFA and blood glucose levels. In the first experiment groups of rats were given bilateral electrolytic LH lesions, knife cuts (K), or control operations and sacrificed 24 hr postoperatively. The KC group had lower levels than either the LH or control groups, however, the KC and LH groups had higher blood glucose levels than the control group. In a second experiment 3 groups of rats similarly were given LH lesions, knife cuts (LH), or control lesions and allowed 2 days to recover feeding behavior. Two additional groups were pair-fed to the LH and KC groups to maintain equivalent body weight. The results showed that these knife cuts placed lateral to the LH additionally block the hyperglycemic response to 2-DG. This unique finding suggests that these knife cuts interfered with the sympathetic activation of the adrenal medulla.


Noradrenergic stimulation of the hypothalamic paraventricular nucleus (PVN) causes an increase in feeding behavior in the rat. The response to this secretagogue norepinephrine (NE) causes as well as after injection of tri- cyclic antidepressants (such as desipramine and amitriptyline) which produce their effect through amine uptake and storage. In the present study, the ascending noradrenergic projections which innervate the PVN and mediate these drug effects were examined using lesions of mesopontomedullary knife cuts. The lesions did not alter the response to 2-DG that was seen in the LH group or the control groups. The present results and previous findings indicate that hypophagia-inducing as well as hyperphagia-inducing hypothalamic damage suppresses the mobilization of FFA, but does not interfere with the hyperglycemic response to 2-DG. The results showed that these knife cuts placed lateral to the LH additionally block the hyperglycemic response to 2-DG. This unique finding suggests that these knife cuts interfered with the sympathetic activation of the adrenal medulla.
HYPOTHALAMIC OBESITY IN RHESUS MONKEYS: GLUCOREGULATORY AND NEUROCHEMICAL COMPARISONS. Joseph W. Kemnitz, Robert W. Goy, and Gary W. Kraemer, Primate Research Center, University of Wisconsin, Madison, WI 53706

Bilateral hypothalamic lesions were produced in three young adult male Macaca mullata. Two sham-lesioned males served as controls. Histological analysis revealed that the lesions were located in the rostromedial lateral hypothalamus. Analyses of regional catecholamine concentrations indicated that norepinephrine (NE) was depleted in the lesioned animals to 46%, 42%, and 34% of control levels in caudate/corpus striatum.

During the year of observation following surgery the lesioned animals became markedly obese. The maximal weights of the lesioned animals were 9.25 kg (104%), 8.75 (93%), and 5.25 (51%) greater than their presurgical weights, while the corresponding values for controls were 1.40 kg (15%) and 2.18 (18%). At autopsy, large masses of fat were noted subcutaneously, intraperitoneally, and pericardially, with no signs of vitally organ function. The obesity was largely dependent upon hyperphagia. The lesioned animals were overeating during the time of supranormal weight gain and they lost weight when food was restricted to caloric levels.

One of the lesioned animals exhibited elevated postprandial serum glucose levels (686 mg/dl vs. 70.5 ± 4.5 for controls) as well as elevated postabsorptive glucose levels (116 mg/dl vs. 65.0 ± 0.0 for controls) and impaired glucose clearance during an intravenous glucose tolerance test after the obesity was established. There was a trend of larger glucose elevations for this animal during an insulin challenge test in this phase of the experiment (8 mg/dl vs. 30.0 ± 11.0 mg/dl for controls and 29.9 ± 13.3 mg/dl for the other lesioned animals). Postprandial and postabsorptive glucose values were more normal (84 mg/dl and 106 mg/dl, respectively) after a 3-week period of food restriction, but were slightly above normal levels throughout the experiment.

The results are consistent with previous reports of obesity following interruption of ascending NE systems in rodents and primates. They further suggest that glucoregulatory deficits are not an invariable consequence of such lesions and that such deficits, when they occurred, may be described as insulin impedance second to obesity.

(Supported by a grant from The Weight Watchers Foundation and NIH grants RO01767, MH08989, and MH21312.)
FEEDING AND DRINKING

717 FUNCTIONAL AND ANATOMICAL STUDIES OF NORADRENERGIC SYSTEM OF THE PERIVENTRICULAR HYPOTHALAMUS THAT CONTROLS FEEDING BEHAVIOR. Sarah P. Lethowig (Rockefeller University, New York, NY 10016).

The paraventricular nucleus (PVN) and periventricular region of the hypothalamus are densely innervated by noradrenergic and serotonergic neurons. This area has been shown to be uniquely sensitive to the feeding stimulatory effect of exogenous norepinephrine (NE) and epinephrine observed in the rat. A series of tests focusing on increasing our understanding of the significance of this phenomenon have yielded the following results.

1) When antidepressant drugs (such as protriptyline and amitriptyline) are injected into the PVN, increased feeding can similarly be seen. This response apparently results from drug-induced release of endogenous NE, as it is selectively blocked by α-adrenergic antagonists, and it fails to occur in animals with a local and selective inhibition of NE synthesis.

2) Electrolyte lesions in the area of the PVN and periventricular hypothalamus attenuate or abolish the feeding response to both NE and the antidepressant drugs.

3) Discrete damage to the PVN also causes hyperphagia leading to obesity, in male as well as female rats maintained on standard lab chow or high fat diet.

4) The increased eating observed with PVN injection of NE is associated with increased drinking of the vagus nerve. Cholinergic vagal afferents to the visera may be involved in this effect, since peripheral injections of the anticholinergic drugs atropine and scopolamine, at very low doses, similarly antagonize the feeding evoked by NE.

5) Caudal hypothalamic and midbrain lesions or knife cuts, which presumably sever PVN efferents to the midbrain, in the medulla oblongata or the vagus complex, have yet to be found to significantly interfere with the NE eating response.

The tests indicate a self-selection feeding paradigm, where dietary protein, carbohydrate, and fat are systematically manipulated, at very low doses, similarly antagonizing the eating evoked by NE.

717 ANTEROVENTRAL THIRD VENTRICULAR REGION (AV3V) LESIONS: THIRST DEFICIENCY TO BETA-ADRENERGIC RECEPTOR ANTAGONIST STIMULATION. R. W. Lind and A. K. Johnson. Department of Psychology and Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Recent work from this laboratory has demonstrated that lesions of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) produce acute adipsia without a compensatory anhydrosis. This observation allows the investigation of the effects of lesions in the AV3V. The results of the present study are as follows.

1) A series of lesions were performed in the AV3V. These lesions were performed using electrolytic lesions as well as a variety of other methods. The results of these lesions were compared to control rats that were not lesioned.

2) Electrolytic lesions in the area of the PVN and periventricular hypothalamus attenuate or abolish the drinking response to AII and HTS. Rats were pretested with 3H2O, either intracardially or intraperitoneally, and the uptake of the 3H2O was measured. The results of these experiments support the interpretation that responses to both of these dipsogens are mediated by the AV3V.

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Male weanling rats (75-100 g), under sodium hexobarbital anesthesia, were lesioned (1.5 m A anodal d.c. for 10 s) at ventromedial hypothalamic (VMH) coordinates (AP: 1.5; L: 1.5; V: 1.5). Lesions were performed 1 mm beneath the surface of the brain. The rats were injected IV with 1.75 mCi 3H2O either immediately following the lesion or 2½ hrs following the lesion and then killed 1 hr post-injection. Food and H2O were unavailable during the experiments. 3H2O served as a tracer for the labeled compound. Incorporation of glycine and glycerol concentration of liver and carcass as well as plasma glucose concentration (PGB) were determined along with the following activity (SA) of these substances and the SA of plasma H2O.

The results demonstrate that within 1 hr post-VMH lesion, PGB is increased 25% (p<.01) and glucogenosis by 51% (p<.05) while lipogenesis is decreased 17% (p<.02). Between 2½-3½ hrs post-VMH lesion PGB approaches control values (+15%, NS) as incorporation of tracer into liver (+70%, p<.05) and carcass (+15%, p<.05) glycerol and liver lipids (+40%, p<.05) is accelerated. 5-6 hrs post-VMH lesion, PGB continues the tendency to be elevated but does not differ significantly from control levels. Glucogenosis is increased 11.5%, p<.02 as are carcass and liver lipogenesis (+25%, p<.05) respectively. Our hypothesis is that VMH lesions cause an immediate and transient sympathetic discharge promoting glucogenosis and suppressing lipogenesis. This is followed by increased uptake of plasma glucose by tissues until uptake begins to lower PGB. At this stage the glucogenosis is increased to maintain PGB.

These data indicate that complex metabolic disturbances occur after VMH lesioning and that the understanding of these disturbances remains to be determined whether these processes seen in the weanling rat also occur in the mature VMH-lesioned rat and whether they underlie the hyperphagia exhibited by the latter preparation.

We investigated whether sodium influences the actions of AII on its receptors on the brain in vivo. Rats were maintained on a normal (controls) or low sodium diet for 2 weeks and received demineralized water ad lib. For intracerebroventricular injections (i.v.t.), we implanted a cannula into the lateral cerebral ventricle. (1) Drinking was induced in the rats by i.v.t. injections of AII (400 pmol), carbachol (400 pmol) and hypotensive (9.7 vs 9.2 ml/30 min, p<0.01; n:20) and there was no difference following carbachol (9.7 vs 9.2 ml/30 min, n:19). (11) The pressor effects of AII and carbachol were tested in conscious, unrestrained rats with chronic arterial catheters to monitor blood pressure (BP). Drugs were applied i.v.t. in a volume of 3 µl. BP increases following AII in the signified rats (n:8) were compared to controls (n:8) at doses of 10 pmol (8.1 vs 11.3 mm Hg, 100 pmol (13.3 vs 16.3 mm Hg), 1000 pmol (14.6 vs 19.4 mm Hg), and 3000 pmol (17.3 vs 25.0 mm Hg). These results show that the pressor effects of AII are reduced in sodium deficient rats. This may be due to changes in the binding properties of AII to its brain receptor. AII has a physiological stimulation of the hypothalamus with the regulation of water intake. It is evident from the present experiments that the potency of AII to elicit drinking depends on the state of sodium balance and this applies to its effect on central mechanisms of BP regulation.


The vasomotor hypothesis states that angiotensin II (AII) is dipsonic because of special sensitivity within the brain to AII's vasostatic action. All presumably vasocostracts the vascular bed in all dipsonic site and exerts an effect on chemoreceptors which drive drinking behavior. The hypothesis is based on the inhibitory effects of AII drinking caused by central administration of substances which bond chemoreceptors (Nicolaidis and Frittsmone, C. R. Acad. Sci. 281, 1975; Kenney and Epstein, JCPP, 92, 1978). In this study we measure the effects of AII to reduced blood pressure and blood flow to infer what happens to resistance (i.e. vasocostriction). Sprague Dawley male rats were used for subjects. The autoradiographic method was developed by Pare, J. and Gorski, Brain Res. Bull. 3:549, 1978). These estrogen induced increases in a.) the number of meals (NM) during light, b.) intermediate interval (IMI) during dark and c.) average meal size (MS) and duration of feeding were compared to baseline values following i.v. injections of AII (30-100 pmol) and carbachol (10-100 pmol). The overall mean values of NM, IMI and MS were significantly reduced following AII (4.8 vs 3.1, 10-130 min, p<0.001; n:18). However, the magnitude of the changes suggest metabolically stimulated increases in blood flow, as well.

Supported by American Philosophical Society, Sloan Foundation and RO15464-17.


Almost all of the diverse actions of beta-endorphin(βE) are consistent with the theory that this hormone produces a widespread state of adaptation that conserves energy expenditure, thus prolonging survival during famine. Many forms of exercise cause an increase in the release of βE. The actions of βE include the inhibition of feeding and drinking, a decrease in electrolyte and water excretion, and the stimulation of fat and carbohydrate storage. βE also acts to inhibit the release of the gonadotropin hormones, thus reducing sexual urges. Carbohydrates can be conserved by βE, as demonstrated by the production of a pre-famine obesity. Obese organisms can survive an extended period of fasting. βE also stimulates the release of anti-diuretic hormone which acts on the kidney to conserve H2O. βE also acts to inhibit the release of the gonadotropin hormones, thus reducing sexual urges. Endogenous naloxone-like peptides may exist for the release of the opioid peptide hormone system and may be involved in the mediation of the effects of βE. In addition to the actions of βE, there are reported to be an increase in the amount of lipids and a decrease in the amount of glucose. These changes in many organ systems suggest a role for βE in the development of a pre-famine obesity. Obese organisms can survive an extended period of fasting. βE also stimulates the release of anti-diuretic hormone which acts on the kidney to conserve H2O. βE also acts to inhibit the release of the gonadotropin hormones, thus reducing sexual urges. Endogenous naloxone-like peptides may exist for the release of the opioid peptide hormone system and may be involved in the mediation of the effects of βE. In addition to the actions of βE, there are reported to be an increase in the amount of lipids and a decrease in the amount of glucose. These changes in many organ systems suggest a role for βE in the development of a pre-famine obesity. Obese organisms can survive an extended period of fasting. βE also stimulates the release of anti-diuretic hormone which acts on the kidney to conserve H2O. βE also acts to inhibit the release of the gonadotropin hormones, thus reducing sexual urges. Endogenous naloxone-like peptides may exist for the release of the opioid peptide hormone system and may be involved in the mediation of the effects of βE.
MORPHOLOGICAL EFFECTS OF INTRAHYPOTHALAMIC KAINIC ACID. Electrolytic lesions, the present morphological study suggests deficits in feeding and drinking identical to those produced by years ago. Whereas intrahypothalamic KA injections produce subthalamus is important to production of the behavioral syndrome.

Lateral injections. The bilaterally injected group which did not duplicates the aphagia and adipsia produced by electrolytic lesions in the same region (Stricker et al., Brain Res., 158: 470, 1978). Since KA may affect ingestive behaviors (Stricker et al., Sch. Med., La Jolla, CA 92037). The results of these experiments suggest that estrogenic metabolites of TP may act on different sites to reduce food intake and body weight. In addition to a direct effect on the hypothalamus, estrogens may reduce body weight gain by inducing changes in adipose tissue metabolism, including lipoprotein lipase activity. These mechanisms may underlie the reductions in body weight, food intake, and carcass fat content seen in gonadally intact, sexually active male rats. Supported by NINDS grants NS-10873, NS-00090, NS-05854-01, NIA-AMD grant AM02785 and NIH traineeship MH11823.

MORPHOLOGICAL EFFECTS OF INTRAHYPOTHALAMIC KAINIC ACID. The anatomical substrate of the LH syndrome has not been well elucidated even though the syndrome was described more than 20 years ago. Whereas intrahypothalamic KA injections produce deficits in feeding and drinking identical to those produced by electrolytic lesions, the present morphological study suggests that damage to nuclear regions in the lateral hypothalamus and subthalamic is important to production of the behavioral syndrome. (Supported by USPHS Grant MH-32410.)


Genetically obese mice (obob mutation) have significantly elevated food intakes and hypothalamic norepinephrine (NE) levels compared to those of lean controls (Olomstead, R. O., Fed. Proc., 36: 5165, 1977). From the results of these experiments we conclude that AMP treatment has a comparable anorectic effect in lean and ob/ob mice in the immediate post-injection period. This anorectic action is paralleled by comparable reductions in NE levels in both groups. Subsequent to this period, anorexia is normal in both saline-treated and AMP-treated lean mice, but AMP-treated ob/ob mice continue to eat significantly more than AMP-treated lean mice. Supported in part by BRSG Grant RR5366 from NIH.

EVIDENCE FOR THE PRIORITY OF A KINAL RELATEO MEDIATION IN THIRST INDUCED BY ISOPROTERENOL TREATMENT. R. Rettig and A. K. Johnson. Dept. Psychology and Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Two experimental groups and 2 control groups were formed. Rats in group 1 and 2 (m7/gp) were subjected to bilateral nephrectomy, while group 3 and 4 (20-35 g) received uretic ligations. Three to 4 hrs after surgery a drinking test was conducted. ISOP (40 mg/kg) was infused for 30 min. The results indicated that the RAS plays a permissive role by allowing the animal to maintain a sufficiently high BP, while the main stimulus for the drinking is hypothalamic input from baroreceptors (Stricker, Fed. Proc., 37:2704, 1978).

Rats having 1 or 2 ipsilateral hypothalamic electrodes that elicited gnawing, eating, and drinking received intermittent electrical stimulation for 45 min following i.v. injection of [14C] deoxyglucose, and their brains were processed according to the method of Sokoloff for determining regional differences in glucose metabolism. To maximize detectability of elicited activity, baseline metabolism was reduced by light barbiturate anesthesia. The stimulation produced predominantly ipsilateral increases in glucose consumption in an extended sequence of structures extending anteriorly from the lateral hypothalamus into the diagonal band of Broca and the septal area, and posteriorly into the central grey, zona incerta, ventral tegmental area of Tsai (VTA), medial raphe, a portion of the tegmental dorsal to the brachium conjunctivum (BC), parabrachial nuclei, and locus coeruleus. Many areas were similarly affected in control rats by hypothalamic stimulation that did not elicit the three behaviors. The largest differences between experimental and control rats were in the lateral VTA, supra-BC tegmentum, and dorsal and ventral parabrachial nuclei. These and possibly a few other marginal structures appear to be the most likely candidates for the destinations and/or pathways of hypothalamic effectors producing gnawing, eating, and drinking.


Male golden hamsters preferentially consume alcohol solutions (< 10% w/v) when given a free-choice between water and the alcohol solution. The pineal gland has been implicated as influencing the predilection for the ethanol solution. Male golden hamsters were allowed to consume an alcohol solution for 72 hr. Light-deprived, sham-pinealectomized hamsters drank more ethanol solution (38 ml/day) than did light-deprived pinealectomized hamsters (31 ml/day; p < 0.001). Sham-pinealectomized hamsters maintained in a long photoperiod consumed more alcohol solution (35 ml/day) than did their pineal-ectomized counterparts (30 ml/day). Serum alcohol levels were higher in sham-operated animals and lower in pinealectomized animals. In subsequent experiments, melatonin, a pineal hormone, was administered either daily for 1 week as a subcutaneous injection (25 μg/animal) or weekly as a subcutaneous beeswax implant (1 mg melatonin/24 mg beeswax) for 5 weeks to hamsters allowed a free-choice between water or a 10% ethanol solution. Food, water and alcohol consumption was measured on a daily basis. Animals treated daily with melatonin consumed slightly less ethanol (2.5 ml/day) than did animals not given melatonin (18.0 ml/day). However, in light-deprived animals given chronic implants of melatonin, alcohol consumption was reduced (27.3 ml/day) when compared to alcohol consumption by light-deprived hamsters not receiving melatonin (34.4 ml/day). Total fluid consumption was also reduced by melatonin treatment (30.7 ml/day) and was greater in hamsters which did not receive the melatonin implants (40.7 ml/day). Melatonin treatment resulted in reduced daily total fluid intake as well as ethanol consumption in light-deprived hamsters. The results indicate that the pineal gland may influence the preference for alcohol consumption in the hamster, but the data suggest that the pineal gland does not alter the propensity to consume alcohol.

(Supported by a grant from the North Carolina Alcoholism Research Authority.)

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Bilateral parasagittal knife cuts in the medial hypothalamus of adult female rats produced overeating and obesity. The Cut rate also drank more of a 20% sucrose solution than did sham controls during 1-hr/day tests. Pretreating the rats with atropine methylnitrate (5 or 10 mg/kg) did not reduce their sucrose intake, and the Cut rats continued to drink more sucrose than did the controls. Atropine reduced their 24-hr fluid intake, although the Cut rats still ate more than did control subjects. The results suggest that cholinergically-mediated visceral responses are not essential for knife cut-induced sucrose overconsumption. In a second experiment subdiaphragmatic vagotomy (Vag) or sham vagotomy (Sham) were performed in knife cut (Cut) and control (Con) female rats. Vagotomy reversed the hyperphagia and obesity of the Cut rats, and also blocked their overconsumption of 20% sucrose solution during 1 hr and 24-hr/day tests. The atropine results of Exp 1 suggest that the vagotomy effect on sucrose consumption may be due to the disruption of vagal afferent activity, or atropine-resistant vagal afferent activity. In a third experiment the subjects of Exp 2 were given ad libitum access to palatable foods (high fat diet, sweetened milk, chocolate-chip cookies) in addition to chow for 10 days. At the start of the experiment the Cut-Vag and Con-Vag groups weighed less than did the Con-Sham group, which weighed considerably less than did the Cut-Sham group. All rats gained weight on the palatable foods, but the Cut-Vag and Con-Vag groups gained the most (260 g), while the Con-Sham group gained the least (85 g). The Cut-Sham group gained more than did the Con-Sham group (205 vs 145 g). Interestingly, both the Cut-Vag and Con-Vag groups consumed significantly less of the sweetened milk diet than did the Cut-Sham and Con-Sham groups, which is consistent with the suppressive effect of vagotomy on sucrose solution intake observed in Exp 2. When returned to the chow only diet all groups lost weight, but the Cut-Vag group lost the most (193 g/10 days). These results demonstrate that while vagotomy reverses the sucrose overconsumption induced by hypothalamic knife cuts, it does not block the hyperphagia and enhanced weight gain displayed by knife cut rats offered access to palatable foods.

(Supported by NIH Grant AM 23064 and CUNY Grant RF 11802)
DRINKING TO CAVAL LIGATION FOLLOWING ABLATION OF PERIVENTRICULAR TISSUE SURROUNDING THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) IS SPECIFICALLY CORRELATED WITH THE POSTLESION RESPONSE TO ANGIOTENSIN II (AII) AND HYDROXYPROPYL-β-CYDROXYGLUCOSPONIC (HYP) IN RATS.

Rats were tested to a range of subcutaneously (s.c.) administered doses of AII (0.5, 1.0, 2.0, 4.0, 6.0 mg/kg) and of HYP (1-16 cc/kg of 10% NaCl w/w) prior to placement of anodal lesions in the AV3V or sham lesioning procedures. The dose-response curve for AII had an ED50 at 1.0 mg/kg and a maximal response of 7.2 ml (in a 1-hr test) at the 4.0 mg/kg dose. One month post-surgery, drug response testing was repeated. Half of the lesioned and sham lesioned rats then underwent ligation of the inferior vena cava and were observed for their water intakes over the next 4 hr. The remaining animals were bilaterally nephrectomized and 24 hr later, the water intake to 2.0 mg/kg AII was tested. The drinking response over the 4 hr post-ligation was positively and significantly correlated with the postlesion response to AII (r = .686, p<.01). Thus, rats which failed to drink to s.c. AII post-lesion also failed to respond to caval ligation. The lesion post-ligation to AII was not significantly correlated with either the response to AII or to that following caval ligation. The chronic elevation in PRC cannot account for the failure to drink to s.c. AII post-lesion as nephrectomy did not reverse the deficit.

These data support the hypothesis that caval ligation acts to induce drinking via those systems mediating the response to s.c. AII, and further, that the lesion produced disruptions of drinking to both the endogenous and exogenous challenges are independent of accompanying alterations of the peripheral reno-angiotensin system. Histological analysis for an area of critical damage within the AV3V region, which produced AII response deficits, implicated a portion of the median preoptic nucleus.

FEEDING AND DRINKING

SELECTIVE GASTRIC VAGOTOMY DECREASES THE SATIETY EFFECT OF CCK-8; and vagal afferents relay the satiety signal to the brain. Supported by NIH Grants MH15455, AM17240 and MH00149.

Large bilateral lesions of the amygdala and ablation of the dorsal hippocampus, including the fornix, significantly enhanced nocturnal drinking and resulted in a tendency toward accelerated re-entrainment following a 12-hr phase shift of the light-dark (LD) cycle. None of the rats were in permanent estrous or anestrus. Knife cuts of the fornix resulted in highly irregular transients after the phase shift of the LD cycle and significantly increased the number of days required to re-entrain drinking rhythms. Since drinking rhythms appeared to free run normally in constant light, the impairment in phase shifting is probably not a deficit in the circadian regulation of drinking. Furthermore, since ablation of the fornix did not produce this deficit, it appears that some other structure damaged by the knife cuts is responsible. All rats in the fornix and hippocampal lesion groups displayed a change in the pattern of drinking bouts for the duration of the experiment but average daily water intake was not affected. It was concluded that the major extrahypothalamic limbic structures may play a modulatory role in the drinking of rats but are not essential to the generation or entrainment of the circadian rhythm in water intake. (Supported by NSF Grant BNS 78 2497 and NIH Grant MH 11218.)

Supported by NIH Grants MH15455, AM17240 and MH00149.
FEEDING AND DRINKING

ANTICIPATION OF 24 HR FEEDING SCHEDULES IN RATS WITH LESIONS OF THE SUPRACHIASMATIC NUCLEI AND HYPOPHYSECTOMY. Jennifer M. Swann* and Friedrich K. Stephan, Dept. of Psychology, Florida State University, Tallahassee, FL 32306.

We have shown previously that rats with lesions of the suprachiasmatic nuclei (SCN) anticipate restricted access to food by an increase in wheel running as reliably as intact rats and present evidence that this behavior shows some of the basic properties of a circadian rhythm, despite the absence of circadian rhythms in intact and drinking and wheel running conditions. Anticipatory activity was not prevented by adrenalectomy (Stephan, Swann and Siik, Behavioral and Neural Biology, 1979, in press). To elucidate the possible role of the pituitary gland in anticipatory activity, hypophysectomized rats and controls were maintained in constant light with ad lib. access to food and water for 10 days, followed by a restricted feeding schedule (one or two daily access periods) at 24 hr intervals for 20 consecutive days. Under ad lib. conditions, both groups showed free running activity rhythms with a period of approximately 25 hr. Under restricted access conditions, the dominant period of activity became exactly 24 hr in both groups, although a weaker activity component with a period longer than 24 hr persisted in some animals. However, while controls showed distinct anticipatory activity within 2-5 days after initiation of the restricted feeding schedule, hypophysectomized rats required 10 days or longer before anticipatory activity appeared. Furthermore, the amount of anticipatory activity was significantly reduced compared to controls. Preliminary data on hypophysectomized rats with SCN lesions indicate that these rats are still capable of anticipating restricted feeding schedules. While some rats no longer showed anticipatory wheel running, simultaneous collections of drinking records of wheel running indicated the presence of anticipatory licking. These results show that restricted access to food at 24 hr intervals synchronizes activity of hypophysectomized rats and that anticipatory activity is reduced, but not prevented, by hypophysectomy. Furthermore, SCN lesions in hypophysectomized rats may abolish anticipatory wheel running but not anticipatory drinking. Thus, the locus of control for anticipatory behavior remains to be determined.


It is well known that some species, such as dogs, sheep and goats, are able to retrieve a water deficit due to deprivation within the first few min. of access to water. Since, in dogs, drinking is complete long before ingested water alters plasma tonicity or volume, the mechanism by which animals take water intake to deficit must rely on other different information. Stimulation which elicited thirst usually also elicited ADH secretion. Therefore, we questioned if ADH secretion was reduced before restoration of plasma tonicity in water-deprived dogs. A preliminary experiment on four dogs, dehydrated for 24-hours, indicated that plasma ADH fell before a detectable change in plasma sodium or osmolality occurred. The two most likely stimuli which could account for this phenomenon are drinking-induced oro-pharyngeal stimulation or gastric distention. In order to test these two stimuli, a second population of dogs was prepared with permanent gastric fistulas, thus allowing either immediate removal of ingested water, or loading of water directly into the stomach. Blood was sampled before dehydration, after 24-hours of dehydration, and at 3, 6, 9, 12, 15, 30 and 60 min. after allowing access to water. Three experiments were carried out: (1) oral rehydration with water available to satiation; (2) oral rehydration coupled with simultaneous removal, via the fistula, of the ingested water; and (3) gastric loading of water, via the fistula, the amount being determined from (1) above. Dogs allowed to drink ad lib were satiated within 6 min. and showed a significant reduction in plasma ADH before a detectable change in plasma sodium or osmolality occurred. While water loading at 9 and 15 min. later showed a weaker activity component with a period longer than 24 hr, plasma ADH was significantly reduced before restoration of plasma tonicity in water-deprived dogs. Supported by NIH Grant AM 6704.


The role of insulin in evaluation-normal, male rats using the osmotic minipump to elevate endogenous insulin levels. Normal-weight animals reduced food consumption by lowering meal sizes without any change in meal frequency when 1.92 U regular insulin was delivered via minipump. Meal sizes averaged 21% smaller with 1.92 U insulin compared to isotonic saline delivered similarly. The purpose of this study was to determine the effect of normal-weight insulin normal-weight insulin on insulin sensitivity. Three hundred and fifty-eight neonatal rats selected from 44 litters of different ages were used in four experiments to investigate the development of behavioral response to a satiety hormone cholecystokinin (CCK). The CCK-induced satiety was first seen in 9-day-old rat pups followed by a disappearance of the effect at day 10 through 12 and then permanently reappeared at day 13. This phenomenon was replicated at the critical ages of day 9 through day 13. Thyroid hormones were returned to pre-pregnation levels, this drinking suggests that oro-pharyngeal stimulation is required for complete satiety. These data indicate that oro-pharyngeal influences, but not gastric distention, are important for CCK-induced satiety in water-deprived dogs. Supported by MHR Grant AM 07529.


Three hundred and eighty-eight neonatal rats selected from 44 litters of different ages were used in four experiments to investigate the development of behavioral response to a satiety hormone cholecystokinin (CCK). The CCK-induced satiety was first seen in 9-day-old rat pups followed by a disappearance of the effect at day 10 through 12 and then permanently reappeared at day 13. This phenomenon was replicated at the critical ages of day 9 through day 13. Thyroid hormones were returned to pre-pregnation levels, this drinking suggests that oro-pharyngeal stimulation is required for complete satiety. These data indicate that oro-pharyngeal influences, but not gastric distention, are important for CCK-induced satiety in water-deprived dogs. Supported by MHR Grant AM 06704.
CIRCADIAN DRINKING RHYTHMS AND MODULATION BY EXOGENOUS LIGHT.

Physiological and behavioral studies have shown that the light-dark cycle exerts a pronounced exogenous influence upon endogenous eating and drinking rhythms. The exogenous stimulus of light was used to investigate its effects upon the rhythmic pattern of water intake as well as the total daily consumption, low, medium, and high intensity light levels. These rhythms, which were used in a series of three experiments to study the interaction of endogenous and exogenous mechanisms in three strains of rats, Wistar (WS), Long-Evans (LE), and Charles River (CH). In experiment 1 with constant light of the three intensity levels, WS and CH rats reduced daily water intake significantly (p<0.01). LE rats, however, did not exhibit a significant decrease in water intake, even at high light intensities. Nocturnality of drinking (max/min daily intake) was reduced in both CH (p<0.01) and WS (p<0.02) after exposure to a light-dark cycle with low intensity light periods in experiment 2. With high intensity light periods nocturnality increased in these two strains (p<0.01). Again, LE rats did not exhibit a significant modification of drinking in response to light intensity manipulations in the light periods. In experiment 3 the superior accessory (SAOT) and primary optic (ROT) tracts were lesioned to evaluate the possible influence of the accessory optic system on the rhythmic pattern of water intake. When compared with non-lesioned controls for each respective strain, LE rats reduced daily water intake at medium (p<0.01) and high (p<0.05) intensity levels of constant light. These reductions were comparable to those exhibited by WS and CH rats (p<0.02). The results suggest that in the LE rats, the SAOT is able to compensate for the effects of light intensity on water pre- suming that the homoeostasis of fluid balance as well as nocturnal drinking patterns. Furthermore, the results demonstrate the interaction of exogenous factors with the rhythmic controls of drinking in the rat.


Infant albino rats (males and females) sustained electrolytic lesions of the periventricular tissue surrounding the antero-ventral third ventricle (AV3V) at 10 days of age. Following weaning at 25 days of age, and throughout development, drinking behaviors were measured daily. Also measured were various behaviors following specific dipogenic treatments, as well as various blood and urine variables. Daily ad lib intakes of AV3V rats were elevated over control intakes from the first measurement period and thereafter. Female AV3V rats were reliably hyperdipsic by 25-30 days of age, and males were reliably hyperdipsic by 15-20 days of age. The daily ad lib hyperdipsia was maintained through 200-250 days of age for both sexes, and reached 216% of control intakes. When the rats were tested for drinking behaviors following specific manipulations, the AV3V rats (of both sexes) were hyperdipsic in response to hypovolemia produced by polyethylene glycol injections; they were hyperdipsic following 24 hour water deprivation. More noteworthy, because of the contrast with rats sustaining AV3V lesions in adulthood, the rats sustaining AV3V lesions in adulthood also showed a significant reduction in water intake, even at high light intensities.

The nucleus of the solitary tract receives gustatory and visceral information from gastrointestinal and cardiovascular baroreceptors and may play an important role in mediating this response. In one group of adult female sheep (1; 7 experiments on 5 animals), removal of oesophageal or abdominal vagal afferents did not significantly alter the drinking pattern. Isotonic fluid was flowed through a Gastro dialyzers and an extracorporeal blood pumping system. Filtration occurred over a 24 hour period in conscious, food- and fluid-deprived sheep. Prior to filtration, they were hyperdipsic during 24 hour food deprivation. The AV3V rats displayed ability to reduce urine volume and increase water intake in response to hypertonic NaCl solution injection above that of controls. However, the AV3V rats were hyperdipsic, and even with an elevated urine output, the rats did not exhibit more body water loss. This water retention was associated with serum hyponatremia for AV3V rats under ad lib conditions. These results suggested a possible disruption of water-savings mechanisms; thus, the rats were water deprived for 24 hours. The AV3V rats displayed ability to reduce urine volume (>64±74%) and to increase urine concentration (>300±200%) during 24 hour water deprivation. Thus, the hyperdipsia of the present AV3V rats seems primary, and the etiology of the hyperdipsia is under study. Supported by: NICHD (HD-08504) and OURC (CR-520).

WATER INTAKE AFTER INTRAVENTRICULAR DEPLETION IN SHEEP: EFFECTS OF CROSSTRAINING THE LEFT AND RIGHT ATRIA. Mark B. Feinstein, Edward F. Stricker and Edward H. Blaln*. Merck Institute for Therapeutic Research, West Point, PA 19486, and Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Reduction of the intravascular fluid volume has been shown to induce water ingestion in a variety of mammalian species. The present experiments provide the first demonstration that cardiovascular baroreceptors play an important role in mediating this response. In one group of adult female sheep (1; 7 experiments on 5 animals), removal of oesophageal vagal afferents did not significantly alter the drinking pattern. Isotonic fluid was flowed through a Gastro dialyzers and an extracorporeal blood pumping system. Filtration occurred over a 24 hour period in conscious, food- and fluid-deprived sheep. Prior to filtration, they were hyperdipsic during 24 hour food deprivation. The AV3V rats displayed ability to reduce urine volume and increase water intake in response to hypertonic NaCl solution injection above that of controls. However, the AV3V rats were hyperdipsic, and even with an elevated urine output, the rats did not exhibit more body water loss. This water retention was associated with serum hyponatremia for AV3V rats under ad lib conditions. These results suggested a possible disruption of water-savings mechanisms; thus, the rats were water deprived for 24 hours. The AV3V rats displayed ability to reduce urine volume (>64±74%) and to increase urine concentration (>300±200%) during 24 hour water deprivation. Thus, the hyperdipsia of the present AV3V rats seems primary, and the etiology of the hyperdipsia is under study. Supported by: NICHD (HD-08504) and OURC (CR-520).


The nucleus of the solitary tract receives gustatory and visceral afferents arising from the facial (VII), glossopharyngeal (IX), and vagus (X) nerves. Extracellular neuronal recordings have been carried out in this nucleus only as a function of sapid, thermal, and tactile stimulation of the tongue.

Gustatory information is essential for the neural mechanism of taste aversion and for modulating hunger-satiety levels. We know that rats have an extraordinarily well-developed mechanism of bait shyness. Our experiments have consistently shown that naive rats will avoid alcohol ingestion. However, forced intubation will yield a chronically dependent rat.

We are investigating this disparity by recording extracellular single unit activity from the nucleus of the solitary tract. Correlations are being made between the naive and the chronic rat and their respective neuronal response patterns. Furthermore, there is an integration of gustatory neural coding with visceral neural coding arising from vagal afferents synapsing on the nucleus. We have found evidence substantiating differential gastric sensibility (J.A. Deutsch, M.C. Young, and T.J. Kalogeris, Science 201, 165 (1978)). We are continuing investigations into this total quality coding of alcohol and other nutrients. Extracellular recording unit activity correlated with gustatory and visceral parametric manipulations. Full results will be presented at the meeting.

This work was supported by grant AA 07129-04 to J.A. Deutsch.
HYPOTHALAMUS
HYPOTHALAMUS


Single unit recordings were made from central neurons in anesthetized male hooded rats. In some animals the effects of lateral hypothalamic stimulation on lateral preoptic area-medial forebrain bundle (LPA-MFB) unit activity were determined. In other animals the effects of LPA stimulation on lateral hypothalamic-medial forebrain bundle (LH-MFB) neural activity were determined. Recordings were made ipsilaterally to the stimulating electrode. A stable baseline discharge frequency was established and single pulses, 0.5 msec in duration, and/or trains of pulses were applied to the stimulation site through a concentric bipolar electrode. Both frequency, usually 0 to 20 Hz, and voltage 0 to 10 volts, were varied in a random manner. The reliability of effects was established by repeated testing. The results of single pulse stimulation on neural activity were examined on a storage oscilloscope and photographed. The results of trains of pulses on neural activity were illustrated graphically by means of a strip chart recorder. Frequency and voltage response relationships were established. Both excitatory and inhibitory effects were observed. Analysis of single pulse stimulation indicated that these effects were monosynaptic. The effects occurred with relatively short latencies and low voltages indicating that interconnections between the LPA and LH exist.

HYPERPHAGIA AND OBESITY FOLLOWING LESIONS OF THE MEDIAL HYPOTHALAMUS IN MAN. Jastrow, R. Oleson, Carol R. Archer* and Byung D. Chung*. Veterans Administration Medical Center and St. Louis University, St. Louis, MO 63104.

Food intake is regulated by the hypothalamus. Bilateral destruction of the ventromedial nucleus (VM) in animals induces abrupt onset of voracious eating while bilateral destruction of the lateral hypothalamus at the level of VM causes cessation of eating. Similarly in man, lesions of the lateral hypothalamic causes anorexia and emaciation; in contrast the effects of VM lesions are more controversial. A 28-year-old man developed progressive left hemiparesis followed three weeks later by face and trunk cutaneous vasodilation and hyperphagia. He began eating five to six meals per day and constantly felt hungry, he gained thirty-five pounds in sixty days. Computerized tomography with coronal and sagittal reconstruction revealed a neoplastic lesion involving the right lenticulostriate nucleus, the right internal capsule, crossing the midline via the hypothalamus and at lesioning the precentral lenticular nucleus. Endocrine studies including TSH, growth hormone, prolactin and cortisol serum levels were normal. Hyperphagia and concomitant obesity was presumed due to bilateral destruction of VM, cutaneous vasodilatation was related to involvement of preoptic area.

It is suggested that in man a medial hypothalamic syndrome is characterized by hyperphagia, obesity and cutaneous vasodilatation. Additional signs may be hyperthermia and diabetes insipidus.
Cells in the ventrolateral area of the ventromedial nucleus in the hypothalamus have a high affinity for estradiol. We are studying the cell architecture and cell chemistry that differences exist between such cells and other neurons, and to look for estrogen effects.

Estradiol benzoate was injected daily subcutaneously in one group of ovariectomized rats. The control group was injected subcutaneously with the vehicle, sesame oil. After 20 days, exposure to estrogen, the experimental rats showed a strong lordosis reflex response and at this time the rats were sacrificed by anesthetizing with nembutal and perfused with fixative according to standard electron microscopy procedures (Peters, 1970). Three regions of the brain were dissected from each rat: the ventrolateral and dorsomedial subdivisions of the ventromedial nucleus of the hypothalamus and, as a control, the ventromedial thalamus. These areas were embedded and thin-sectioned for viewing with the electron microscope.

The overall morphology of the ventromedial nucleus consists mainly of neurons and a few interspersed glial cells. The neurons contain large oval nuclei, the nuclear membrane of which is often invaginated; the nucleolus is finely granular with a densely granular matrix. The axons of these cells are classified according to their function and their efferent pathways. The axons of some cells project primarily to the cerebral cortex, while others project to the thalamus, hypothalamus, or brainstem. The axons of other cells project to various parts of the brain, including the basal ganglia and the cerebellum. The axons of still other cells project to the spinal cord, where they synapse with motoneurons. The axons of some cells project to the retina, where they synapse with photoreceptors. The axons of other cells project to the spinal cord, where they synapse with motoneurons.

Recent Golgi and electron microscopic evidence has revealed that dendrites of hypothalamic origin extend into the optic and suprachiasmatic nuclei. In a series of anatomical studies with anterograde tracing methods, we now report that these dendrites receive both periventricular and retinal inputs in the hypothalamus. Injection of (3H) proline into the optic and suprachiasmatic nuclei of three rabbits, projections to the hypothalamus included the suprachiasmatic nucleus and a few scattered labeled axons dorsal to the optic tracts. We injected horseradish peroxidase (HRP) into the superior colliculus of three rabbits and demonstrated, electron microscopically, that dendrites of hypothalamic origin extend into the optic and suprachiasmatic nuclei, and that these dendrites receive both periventricular and retinal inputs in the hypothalamus. The number of silver grains diminished as fibers ascended into the optic and suprachiasmatic nuclei, and that these dendrites receive both periventricular and retinal inputs in the hypothalamus. The number of silver grains diminished as fibers ascended into the optic and suprachiasmatic nuclei, and that these dendrites receive both periventricular and retinal inputs in the hypothalamus.
751 SEROTONERGIC INNERVATION OF THE LATERAL HYPOTHALAMUS: EVIDENCE FROM SYNAPTOSOMAL UPTAKE STUDIES.  J. Heym and W. G. Gladefelter. Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

In rats the lateral hypothalamus (LH) has been shown to contain relatively high concentrations of 5-HT. 5-HT uptake systems, which release serotonin from presynaptic sites and which are involved in processes such as neurotransmission and cell communication, have been studied extensively in the central nervous system. 5-HT uptake is mediated by a high-affinity, low-capacity transport mechanism that is thought to be involved in the regulation of serotonin release and neurotransmission.


The paired suprachiasmatic nuclei (SCN) are thought to regulate timing for a variety of biological rhythms, e.g., the sleep-wake cycle. 5-HT neurons in the hypothalamus are involved in the regulation of circadian rhythms. The SCN contains a high density of 5-HT immunoreactive neurons, and 5-HT innervation to the SCN is thought to be involved in the regulation of circadian rhythms. The SCN is a critical component of the circadian clock, and its function is affected by light exposure. The SCN is also involved in the regulation of other behaviors, such as feeding and sleep-wake cycles. The SCN is a critical component of the circadian clock, and its function is affected by light exposure. The SCN is also involved in the regulation of other behaviors, such as feeding and sleep-wake cycles.

Localized injections of horseradish peroxidase (HRP) were placed in the suprachiasmatic nucleus (SCN) of rats by lontophoresis. Sections ofdehyde-fixed brain were prepared by the benzidine dihydrochloride method for demonstration of HRP and examined microscopically. Neurons showing retrograde labelling with HRP were observed in brainstem and diencephalon following injections completely localized to the SCN.

Labelling of brainstem neurons was observed in the nucleus centralis superior and in the lateral part of the dorsal tegmental nucleus. Labelled neurons were observed only occasionally in raphe dorsalis. In the hypothalamus numerous neurons were labelled in the mediodorsal preoptic area, the anterior hypothalamic area (particularly in the region adjacent to the SCN), the ventromedial nucleus and the periventricular hypothalamic nucleus from its rostral to caudal extent. Scattered arcuate nucleus neurons were labelled. In the thalamus labelled neurons were present in the paraventricular nucleus, nucleus reuniens and the ventral nucleus of the lateral geniculate body.

The material also provided evidence for a projection from one SCN to that on the contralateral side. Even in cases of very small injections into the SCN contralateral labelled neurons were evident in the contralateral SCN and anterograde labelling of axons was evident with the axons crossing the midline beneath the third ventricle and entering the contralateral SCN.

These observations confirm previous reports of projections to the SCN. They further extend information on the innervation of the SCN by demonstrating previously undescribed projections from brainstem, thalamus and hypothalamus and a projection interconnecting the nuclei on each side. Supported by USPHS Grant NS-12267.

COMBINED EFFECTS OF FRONTAL HYPOTHALAMIC CUTS AND PREOPTIC (POA) STIMULATION ON ANTERIOR PITUITARY HORMONE RELEASE. C.P. Phelps and J.A. Colombo, Dept. of Anatomy, College of Medicine, Univ. of South Florida, Tampa, Florida, 33612.

The present set of experiments continues our investigation of the general problem of segregation of functions within the POA-hypothalamic complex related to the control of LH, prolactin (Prol.) and TSH release by the anterior pituitary. Recently we have examined the effects of chronic (90d) frontal retrochiasmatic cuts (FC) on LH, Prol. and TSH release after electrical stimulation of the POA. Stimulation was performed at various sites in the SCN. They further extend information on the innervation of the SCN. These observations confirm previous reports of projections to the SCN by demonstrating previously undescribed projections from brainstem, thalamus and hypothalamus and a projection interconnecting the nuclei on each side. Supported by USPHS Grant NS-12267.

HYPOTHALAMUS


Horseradish peroxidase (HRP) was obtained and, from this material, the afferents to the parabigeminal nucleus going to the superior colliculus and lateral geniculate nucleus were identified. The afferent connections to the parabigeminal nucleus going to the superior colliculus and lateral geniculate nucleus were identified. The afferent connections to the parabigeminal nucleus going to the superior colliculus and lateral geniculate nucleus were identified.

These results are the first demonstration of the afferents to the rat retrochiasmatic area and they suggest that those fibers originating in the parabigeminal nucleus might carry visual information towards the hypothalamus.

Supported by Grant No. MH 28440 to G.W.M. and NIH Training Grant No. MH 14643 (V.S.M.).

Opiates and opiate peptides administered intracranially and systemically modulate neurohypophysial secretion in vivo. To investigate a possible mechanism for this effect we have applied opiates by perfusion to hypothalamic slices in vitro while monitoring extracellular unit activity in the paraventricular nucleus (PVN) of the rat. This nucleus contains cell bodies of vasopressin- and oxytocin- and other peptide-containing (e.g., enkephalin) neurons which project to the neurohypophysis.

Brains were quickly removed from 200 g male Sprague-Dawley rats with or without prior transcardial perfusion of the animal with oxygenated Hank's BSS (4°C) and coronal sections (500μμ) of the diencephalon were cut on a tissue chopper. The slices were placed in a chamber where they were perfused over both surfaces at 1.5 ml/min with 0.9% NaCl, 8.03; KH2PO4 0.4; CaCl2 0.4; glucose 11; phenol red 0.03; NaHCO3 4.2; Na2HPO4·7H2O 0.3; KH2PO4 0.03; CaCl2 0.75; MgCl2·6H2O 0.5; MgSO4·7H2O 0.4; glucose 11; phenol red 0.03). Recordings were pursued in the anatomical area of the PVN localized with the aid of a dissecting microscope. 71 probable PVN neurons in 13 slice preparations displayed spontaneous activity similar to that associated with vasopressin neurons. Low intensity electrical stimulation delivered from bipolar metal electrodes placed on the neurohypophyseal tract elicited antidromic action potentials in PVN neurons.

Responses to opiate application were investigated in 27 PVN neurons. On 15 of these neurons, including 4 phasic cells, neither the level of spontaneous activity nor the pattern of bursting activity were affected by addition of morphine, naloxone or enkephalin to the perfusate (all at 10-6M). On 2 tonically firing neurons, 10-6M morphine caused a decrease in spontaneous activity which, on 7 neurons, was reversed by naloxone (10-4M). These results demonstrate a direct depressant action of opiates on certain cells in the PVN; however, opiate effects on neurohypophysial secretion in vivo may be indirect and dependent on preservation of appropriate neural circuitry.


The afferent projections to the supraoptic nucleus (SON) have been studied with a variety of techniques in several species. Thus far, no anatomical evidence exists for relatively direct projections from the viscerogustatory relay nuclei in the PVN and the SON though clearly, viscerogustatory receptor inputs alter ADH release. Recently investigators have explained viscerogustatory actions of ADH release on the basis of hypothalamic leadings from the solitary nucleus to the paraventricular nucleus, which also releases ADH (Ricardo & Koh; Brain Res., 152:1, 1978). Thus we sought to determine the afferent projections of the SON by lentencroptically injecting minute quantities of horseshard peroxidase (HRP) into the SON and by processing the tissue with the new and sensitive elumes modification of the HRP histochemical method (exp. Brain Res., 29:541, 1978). Using this technique, we find that both the solitary and parabrachial nucleus (but not the ventrobasal thalamus) project to the SON. Projections from other brainstem and limbic structures to the SON were also demonstrated. They included the lateral septum; nucleus of the diagonal band; paraventricular nucleus; periventricular, medial and lateral preoptic nuclei; the bed nucleus of the stria terminalis; subparafascicular organ; the anterior, lateral, periformical, circular, premammillary, posterior and submammillary hypothalamic nuclei; the median eminence; all nuclei and tracts of the hypothalamic nuclei; central grey; dorsal tegmental nucleus; ventral tegmental area; locus coeruleus; dorsal raphe nucleus; nucleus centralis superior; raphe pontis; nucleus reticularis tegmenti pontis; and the nucleus reticularis parvocellularis.

PLACEMENTS OF HRP INTO THE IMMEDIATELY OVERLYING ANTEROLATERAL HIPPOTHALAMUS REVEAL A DIFFERENT AND MORE PROXIMATE PATTERN. It was essentially the reciprocal of efferents to this area described by Saper et al. (J. comp. Neurol., 183:689, 1979). These results indicate that the long and short viscerogustatory projections from the SON are directly to the SON as do many other brainstem and limbic structures involved with autonomic, homeostatic processes. In addition to these results, someshow that the HRP injection revealed the efffrent projections of the SON alone. Axons leaving the SON ascend vertically for about 1 mm into the anterior hypothalamus before turning sideways. Upon contact with the medial accessory accessory, the fibers turn ventromedially toward the infundibulum.

Supported in part by USPHS grants NS7687 to D.N. and NS10928 to L.J.B.
765 LATERAL HYPOTHALAMIC STIMULATION IN HEMIFOREBRAIN ABLATED RATS: A behavioral test with LH stimulation was conducted for the next 20 weeks. Urine deposition is an androgen supported behavior, and declines to about 50% of normal following castration (Price, Hormones and Behavior 6: 395-397, 1975).

In males in which the cuts were at least at the anterior 40% of the anterior hypothalamus and all of the medial preoptic area from the medial forebrain bundle, no copulation was observed even after 20 weeks postcastration. Subcutaneous implants of silastic capsules of testosterone effectively restored copulatory behavior to castrates, but not to the knife cut males. These animals also deposited 50% less urine on the threaded rods (thus resembling castrated males) than did sham operated males. The testosterone implants did not produce any increase in urine marking in these knife cut males, although intact (sham operated) males in control groups showed an increase in urine marking. The cuts placed anteriorly or posteriorly so as to spare as little as 25% of the area lateral to the medial preoptic area or anterior hypothalamic region allowed a nearly normal increase in urine deposition following androgen implant. The cuts appeared to selectively block the influence of androgen on urine marking. Since the cuts caused only a decline to the level of castrates, no influence other than that of androgen was removed, and the medial preoptic region is not an integrative area for urine marking.

In contrast, the cuts did not block an androgen induced weight increase.

Supported by NIMH Predoctoral Fellowship #MH 05423 to CWS.

766 LATERAL HYPOTHALAMIC STIMULATION IN HEMIFOREBRAIN ABLATED RATS: VIGOROUS STIMULATION BEHAVIOR IN RATS AND IS THEREFORE FOUNDED TO BE REWARDING. These rewarding effects can be quantitatively characterized (Gallistel, J. Comp. & Physio. Psych. 92, 1978), but due to the widespread connections of HPB to brainstem and forebrain, anatomic studies are difficult. In a dramatic report, Huston and Borkovsky (Physio. & Behav. 27, 1979) demonstrated that rats with complete forebrain ablations, having the thalamus and hypothalamus as the most rostral structures in the brain, were trained to lever press for LH stimulation. They were then implanted with a bilateral array of LH stimulating electrodes, and were trained to lever press for LH stimulation level were also shown to lead to systematic variations in running speed. These variations occurred in running speed but these variations occurred, in full, on the very next trial. These results are in good agreement with previous findings in intact rats (Gallistel, Stellar, & Bubis, J. Comp. & Physio. Psych. 92, 1979) and suggest that connections between the MFB and its ipsilateral forebrain targets are not necessary for what appears to be normal LH self-stimulation reward.


Different projections from the medial anterior hypothalamus (MAH) were studied in tritiated amino acids injected into the medial basal hypothalamus including the hypothalamic region in guinea pigs. 48-56 hours after injecting tritiated proline the animals were perfused with 10% formalin and processed through a paraffin tissue technique. Spontaneous projections from MAH projected not only to local areas, such as suprachiasmatic n, retrochiasmatic and lateral hypothalamic areas, but also to more caudal targets in the brain. Labelled axons and diffuse grains could be seen in POA and OVLT, and in the diagonal bands of Broca and its horizontal and vertical nuclei. Some of these labelled fibers passed through the diagonal bands of Broca and ascended to the septum. Medial, lateral and triangular septal nuclei also received projections which ascended directly from the medial anterior hypothalamus through POA. Dorsally, through the stria terminalis, the labelled fibers could be followed to the amygdala. Through the stria medullaris, anterior hypothalamic axons reached the lateral habenula and the central gray of the midbrain. Following the periventricular fiber system, the axonal projections from MAH terminated in the periventricular and other midline nuclei of the thalamus, and also distributed to the central gray of the midbrain. Laterally, it projected to the lateral hypothalamus, suprachiasmatic n, substantia innominata and the anterior and medial amygdaloid nuclei. Lateral hypothalamic axons included medial forebrain bundle (MFB) axons labelled heavily. The medial part of MFB was labelled heavier than the lateral part. Posteriorly, MAH projected directly into the medial basal hypothalamus including the ventromedial, dorsomedial and arcuate nuclei. The arcuate nuclei was only slightly labelled. The median eminence was labelled in both internal and external zones. MAH also distributed fibers to the ventral and dorsal premammillary and supraoptic nuclei, and to the posterior hypothalamus. MAH fibers travelled via the capillary plexus of the floor of the third ventricle to reach the central tegmental area of Tsai or secondarily merging with the periventricular fiber system, distributed to the central gray of the midbrain. Dorsally, MAH fibers could be followed to the rostral pons. A few labelled fibers in the MFB projected to the tegmental area of the mediodorsal and pons. Contralateral medial POA, MAH, posterior hypothalamic nuclei, and the heaviest contralateral label was in the pretectal area and ventral and dorsal premammillary nuclei. (Supported in part by NIGMS grant HD 3865).


A descending input from the midline diencephalic areas to the mesencephalic reticular formation has been envisaged to underlie a tonic, reinforcing control of the waking brain. In order to substantiate morphologically this hypothesis and to precisely define the exact source of this descending input, unilateral injections (0.04-0.06 μl) of 50% horseradish peroxidase (HRP) were made spaced to define the cuneiform nucleus and the adjacent central tegmental field of exit. The animals were allowed to survive 48 hours and the sections were treated according to either the diaminobenzidine, or the tetramethyl-benzidine, or the benzidine-dihydrochloride procedure. Immunocytochemical labeling was performed by means of neutral red to distinguish the limits of nuclear groups. Positive cells, with HRP granules in the soma and processes, were consistently found ipsilaterally (and very few contralaterally) at 0.2 to 0.8 mm from the wall of the aqueduct and the third ventricle. Thus labelled neurons were seen in the midbrain periaqueductal gray (mostly in its medio-dorsal aspect), in the periventricular gray medially to the retroflex bundle (contrasting with complete lack of positive cells in the adjacent paraependymal-centromedian thalamic complex), in the periventricular posterior hypothalamic area, and continuing up to the periventricular anterior hypothalamic areas. These results demonstrate that, in addition to a previously described subthalamic input to the midbrain reticular formation (Soc. Neuroc. Abstr., vol. 4, p.49, 1978), a midline system extending from the mesencephalic reticular formation to the midline hypothalamic core, and then to the upper brainstem reticular core. The nature of this descending projection is now being investigated electrophysiologically. Supported by NMS grants MH-3689 and MH-5781.

The results of two groups of experiments suggest that the locus coeruleus is involved in the adrenergic nerve pathways centered in the dorsal motor nucleus of the vagus and adjacent parts of the nucleus of the solitary tract (the dorsal medullary group)1). An electrolytic lesion in part to the terminal area of the hypothalamic paraventricular nucleus (PVH) in the rat. First, electrolytic lesions were placed in the dorsal vagal complex, or knife cuts were made rostral to it in 12 animals. After 1-4 wks, the hypothalamus was processed by Hartman's method for the immunohistochemical localization of dopamine-β-hydroxylase, and damage in the brainstem was assessed in thionin-stained frozen sections. In those experiments with lesions involving the dorsal medullary group, and in animals with deep knife cuts, the density of adrenergic varicosities in the caudal half of the ipsilateral PVH was reduced by about one-third. Second, 10-40 nl of HB (332) was injected stereotactically into the PVH and adjacent regions of 10 rats. After 24-48 h survival times, frozen sections of the hypothalamus and brainstem were processed with TMB histochemistry. In each experiment, retrogradely-filled cells were identified in the nucleus of the solitary tract2) and dorsal motor nucleus of the vagus, and in the LC. This evidence, when considered together with the results of the lesion studies, suggests that adrenergic neurons in the LC and dorsal vagal complex project to the PVH. Previous autoradiographic studies have shown that the LC3) and nucleus of the solitary tract4) project to the PVH, although the neurotransmitter involved in the latter pathway cannot be identified.

In earlier immunohistochemical studies, we showed that oxygen-toxins-stained fibers from the PVH innervate the LC and dorsal vagal complex. Immunohistochemical results discussed here in terms of physiological data which support the hypothesis that the PVH, LC and dorsal medullary group together are involved in neural circuitry that can regulate, via neural mechanisms, as well as well as neural functions within the CNS itself.

This work was supported in part by NIH Grants 1R01 NS-12311, NS-13672, and by a grant from the Sloan Foundation.

References:
1) Swanson, L.W. and B.K. Hartman. J. Comp. Neurol. 163:467 (75)
2) Reis, R.D. and A. Swanson. Brain Res. 127:23 (77)
3) Jones, B.B. and R.T. Moore, Br. Res. 272:23 (77)


The cardiovascular (CV) responses to an acute emotional situation was determined using anesthetized rats with the hypothalami area in question. Three groups of rats were subjected to no stimulus (SN), a 10 min forced swimming in water (WS) and a 10 min exposure to an inescapable shock (SS) in an electric footshock apparatus. After the tests, the hypothalami area were studied with the HRP method in three baboons. Flow probes were aseptically implanted on the renal artery. Two weeks later the animal was anesthetized with ketamine/xylazine (200 mg/g) and an arterial cannula was placed in the femoral artery from which arterial pressure and heart rate were recorded. Using a pericardicographic micropipetaneous procedure, the approach, location in the hypothalamus was identified by lowering a stimulating electrode through a guide cannula and recording the CV responses to electrical stimuli. Of particular interest was that acute freezing (0.1M or 0.2M) were delivered into the right lateral ventricle to test the effects of lesion type and size on activity counts, food and fluid intake at 6 and 24 hr after surgery and had their stomachs examined 24 hr later; the others were given food and saline for 24 hr. Adenosines were used to test the effects of lesion type and size on activity counts, food and fluid intake at 6 and 24 hr and three measures of stomach pathology. Of the nine response variables a significant effect of lesion type (current) obtained only for the total amount of activity at 6 hr and saline consumption at 6 and 24 hr. Single comparisons indicated that LA lesions produced higher activity counts than any other in the first 6 hr post-lesion; in the latter 24 hr, however, LC rats had higher activity counts, and as a result at 24 hr LA and LC groups had high activity counts. Large anode rats drank more than all other groups, but only RV rats drank after saline injection. The only factor which proved significant for all nine variables was size of lesion. Small-lesion groups behaved indistinguishably from control and saline groups, while large-lesion groups showed all rostral-caudal gradient. The results suggest that too great as well as too little an amount of puromycin made available to specific regions leads to less or no impregnation.

Supported by research grant no. NS01960 from NIMH.
Anodal (2mA for 20 sec) or cathodal (2mA for 40 sec) electrolytic lesions were made with monopolar stainless steel electrodes aimed at the ventromedial hypothalamus of rats. Both unilateral and bilateral lesions were performed. Behaviorally these anodal lesions produce hyperactivity within two hrs after the lesion, accompanied by voracious eating or stomach ulcers (Wiener, Nobrega, Ossenkopp, in preparation). Cathodal lesions produce only ulcers.

Three and one-half hrs after the lesion, the animals were injected with 30 µCi of $^{14}$C-2-deoxy-D-glucose. The procedures of Sokoloff were used to obtain auto-radiographs of brain sections from these rats.

Anodal lesions produce profound decreases in labelling in a sphere around the lesion site almost 2mm in diameter. Furthermore there is usually an intense increase in labelling in some specific ipsilateral regions away from the lesion site including, for example, zona incerta, lateral hypothalamus, globus pallidus, and substantia nigra, zona reticulata. The cathodal lesions, although longer in duration, produce a much smaller region of decreased labelling around the lesion site. These radiographic results were compared with the anatomical changes in these sites observed with conventional histology.

The dynamic three-dimensional changes in local brain activity produced by lesions over time can be observed with this method. This promises to help us understand the neural basis of the behavioral syndromes produced by lesions.

(Supported in part by NSERC grant A7077 to J.Y. and NRC grant number 332 to N.W.)
Repetitive firing of bag cells in Aplysia leads to egg laying in vivo and secretion of a peptidergic egg-laying hormone (ELH) in vitro. Extracellular recordings from giant cell neurones have characterized the propagation of spikes during afterdischarge. Variations in amplitude and/or duration of spikes during repetitive firing and the associated changes in depolarization were well-defined. The threshold typically had faster rise times, larger overshoots and more hyperpolarization afterpotentials. Repetitive stimulation led to frequency-dependent inhibition of the cell. FP was usually detectable at 0.5 Hz, and fully expressed between 0.8 - 4 Hz. Accommodation occurred rapidly at 10 Hz. Larger spikes were associated with an increase in duration and the onset of FP. FP resulted from a progressive enhancement of an inflection on the falling phase. A short conditioning train of strong hyperpolarizing pulses caused inactivation of spike generation, which lasted a few seconds. Experiments with 0-Na+ ASW (Tris-substituted) and/or 0-Ca++ (1mM EGTA) ASW revealed that Na+ and Ca++ dominance. Na+-dominant spikes showed less FP. Similar to 0-Ca++ ASW, solutions containing Ca++ and Mn++, reversibly abolished FP. The inflection on the falling phase of the action potential was severely attenuated at higher concentrations. FP was abolished when the spike was severely attenuated. The inflection was augmented in L-mimosine (4mM), which increased the spike duration. Na+ and Ca++ antagonism. These experiments generally support the hypothesis that soma action potentials of bag cells have both Na+ and Ca++ components. It is proposed that FP results primarily from potassium-inactivation which then unasks a calcium current. FP may play a significant role in augmenting ELH release.

Supported by the Connaught Foundation and MRC grants A0395 and A0402.

Two-dimensional gel electrophoresis of neurosecretory polypeptides in the crustacea, C. G. Andrew (SPOR: H. Bann), Biology Dept., York University, Toronto, Ontario, Canada M3J 1P3.

Two-dimensional polyacrylamide gel electrophoresis is capable of resolving hundreds of proteins from microgram amounts of complex biological material. Proteins are focused according to their isoelectric points and by the molecular weight, according to size in the second dimension. Determining the precise location of neurosecretory cell bodies is a prerequisite for studying the synthesis and processing of neurosecretory polypeptides stored in axon terminals. This is comprised of the secretory vesicle of the plasma membrane of the soma. At the soma level, two-dimensional electrophoresis with tetrodotoxin (10-5 M) was used to accommodate the two-dimensional gel electrophoresis. It is proposed that FP results primarily from potassium-inactivation which then unasks a calcium current. FP may play a significant role in augmenting ELH release.

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Kinetics of the slow currents during normal bursting in Aplysia cell R15. William B. Adams and Irwin B. Levinson. Friedrich-Miescher-Forschungsinstitut, P.O. Box 273, CH-4002 Basel, Switzerland.

Bursting activity in cell R15 in the abdominal ganglion of Aplysia is mediated by voltage- and ion-dependent conductances having slow kinetics. During a burst, the cell is depolarized by a flow of inward current. Using voltage-clamp techniques, we have found a transient increase in the inward current flow following each action potential. The increase amounts to 2-4 nA, lasting from 1 to 3 seconds. During temporal summation when action potentials are sufficiently close together, and tends to maintain the burst. In addition to the transient component, a smaller but longer-lasting increase in outward current. The increments in outward current are only a small fraction of a nanomole, but they persist for tens of seconds, and are thought to contribute to the burst. Because of the difference in kinetics between the two current components, the outward current plays a more important role as the burst is over and the outward current is out.

The pattern of burst activity appears to depend on the amplitude and recovery kinetics of these two current components. The normal burst pattern can be altered by dopamine, serotonin, vasopressin and modulators of cyclic nucleotide metabolism. Attempts to elucidate the effects of these agents on the parameters of the individual current components are in progress.

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770 TWO NEUROHORMONES PARTICIPATE IN THE CONTROL OF CIRCADIAN NEURONAL ACTIVITY IN THE CRAYFISH.
It has been postulated that a hormonal channel modulates the excitability of the nervous system of decapod crustaceans along the 24-hour cycle (Aréchiga, H., Fed. Proc. 36:2036, 1977). Neurodepressing Hormone (Neurodepressing Hormone) has been identified in the nervous system and blood of the crayfish, exerting a depressant action on the excitability of an ample variety of neuronal elements (Aréchiga, H., Cabrera-Peralta, C. and Huberman, A., J. Neurobiol., 1979, in Press.) The concentration of this peptide varies along the 24-hour cycle, being highest at day-time.
In experiments measuring the activity of Neurodepressing Hormone (NDH) at different times, it became evident that another substance was present in the nervous system, capable of increasing the spontaneous activity of motoneurons in isolated ganglia. By successive steps of Sephadex G-25 and G-15 chromatography, it has been possible to separate this neuroexcitatory substance, which appears to be a peptide of low molecular weight, eluted in fractions neighbour to those containing NDH in G-15. The greatest amount of this substance is contained in the eyestalk, but lesser quantities are found in the supraoesophageal ganglion and thoracic ganglia. Its excitatory effect does not show noticeable desensitization. The concentration of the eyestalk varies along the 24-hour cycle, attaining a high level at night and decreasing during the day. This rhythm persists in constant darkness in the opposite phase to that of NDH. The rhythm of secretion of both peptides can be phase-shifted by entraining animals to different light-darkness regimes.
From these evidences, it appears that the level of excitability of the crayfish nervous system is modulated in a circadian manner by two peptides with opposite effects.

777 COMPLEX RECEPTOR NEURONS IN TRITONIA: NEURONAL CORRELATES OF A CHANGE IN BEHAVIORAL RESPONSIVENESS.
Teresa Audesirk and Gerald Audesirk. Friday Harbor Labs, Univ. of Washington, Friday Harbor, WA 98250.
When contacted by the tube feet of certain predatory sea stars, the nudibranch Tritonia diomedea performs a characteristic receptor component of an initial swim (Williams, 1967) followed by an "escape run" consisting of rapid crawling locomotion. Although the oral receptors mediate these behavioral changes, it has been hypothesized that ording to stop locomotion and begin to feed, during the escape run the animal will not feed, and in fact behaves as if it cannot tell that food is being approached. The CNS of Tritonia contains a population of complex receptor neurones which are both primary mechanoreceptors and secondary mechanoreceptors and chemical receptors receiving sensory input from the oral veil and anterior foot. These receptors normally respond strongly when the oral veil is touched with a sea whip, Tritonia's normal food source, but during the time of the escape run, these receptors are much less responsive (30%) to food stimuli, although their input from the periphery is not qualitatively different.

During the swim, the receptors are synaptically driven to produce bursts of spikes, in phase with ventral flexions. Three hypotheses can be advanced to account for the responsiveness of the receptors: (1) aftereffects of swim spiking reduce excitability; (2) aftereffects of swim synaptic activity reduce excitability; (3) synaptic inputs during the escape run inhibit spiking. These hypotheses were tested by hyperpolarizing a receptor neuron during a swim to prevent spiking and by driving an "artificial swim" by current injection. It was found that if spikes were prevented during a swim, nearly all of the responsiveness change was abolished. Second, an almost normal responsiveness change was still observed if the artificial swim occurred at a time when the chemosensitive receptors were still active. Finally, if the stimulus was a drug which inhibited neuronal firing by high calcium solutions. These results support the first hypothesis, but minor effects due to the second and third hypotheses cannot be ruled out.

We propose that the responsiveness change is intrinsic to the complex receptors, and is elicited by spiking during a swim. This change in responsiveness may be a possible mechanism by which Tritonia behavior is modified by immediate past experience, contributing to behavioral flexibility.

779 NEUROANATOMICAL LOCALIZATION OF THE SIALIC SALIVARY NEUROEFFECTOR SYSTEM OF KELIOMIS: HETEROGENEITY OF BILATERAL COORDINATING MECHANISMS.
Fred Nails, Stanley B. Lester and Ronald W. Joyner*. Depts. of Physiol. & Biophys. and Zool., Univ. of Iowa, Iowa City, IA 52242.
The salivary neuroeffector system of Keliomis consists of the paired salivary glands and two identified buccal ganglion neurons, 4R and 4L. In contrast to most gastropod neuroeffector systems, both the central and peripheral components of this system are readily accessible to intracellular analyses. Previous work has shown that two different mechanisms contribute to the food extract-induced activation of the salivary glands. First, the two neurons 4 can receive identical synaptic input from higher order buccal ganglion neurons from the same side, the two neurons 4 are electrically coupled, with a mean coupling coefficient of ~0.62 (±28, S.D.+ .16). Activity within each salivary gland is coordinated by effective electrical coupling. However, bilateral coordination of activity between the two salivary glands requires neural control, because there is no communication between the two glands.
We now provide morphological and electrophysiological evidence for a third mechanism for bilateral coordination: innervation of each salivary gland by axons from both neurons 4. Intracellular injection of the fluorescent dye Lucifer Yellow has shown that both neurons 4 can send an axon to both salivary glands. Electrophysiological evidence for dual innervation was obtained in the following manner. When one neuron 4 is stimulated at frequencies ≥ 2 Hz., a delay occurs between the spikes in the two glands. This delay is greatest during the "escape run" consisting of rapid crawling locomotion. Although the oral receptors mediate these behavioral changes, it has been hypothesized that ording to stop locomotion and begin to feed, during the escape run the animal will not feed, and in fact behaves as if it cannot tell that food is being approached. The CNS of Tritonia contains a population of complex receptor neurones which are both primary mechanoreceptors and secondary mechanoreceptors and chemical receptors receiving sensory input from the oral veil and anterior foot. These receptors normally respond strongly when the oral veil is touched with a sea whip, Tritonia's normal food source, but during the time of the escape run, these receptors are much less responsive (30%) to food stimuli, although their input from the periphery is not qualitatively different.

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The synthesis of putative neurotransmitters in the ventral and lateral eyes of Limulus was studied using a modification of the procedure of Hildebrand et al. (J. Neurobiol. 2, 231-246, 1971). Results are summarized in the table below. Ventral eye preparations were dissected into a photoreceptor rich (p-fraction) and a nerve fraction (n-fraction) before extraction and electrophoresis.

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Neurotransmitter</th>
<th>p-Fraction</th>
<th>n-Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline</td>
<td>Ach</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glutamate</td>
<td>GABA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>5-HT</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>NE</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tyramine</td>
<td>Oct</td>
<td>+</td>
<td>+</td>
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</table>

Several interesting observations emerge. 1. The types of putative neurotransmitters synthesized, and the distribution of synaptic activity between all-rich and all-poor neurons are similar in an in vitro and ventral eye preparations. 2. Although serotonin has a number of important pharmacological effects on cells in the Limulus, no serotonin synthesis is detected. 3. Incubations with tyramine show a clear selectivity and accumulation of octopamin cell rich-fraction of both ventral and lateral eye preparations. Octopamine is a possible neurotransmitter or neurohormone in these preparations. Further support this to this idea comes from observations that both ventral and lateral eye prep. contain significant octopamine (6.02 and 32.8 p moles per mg tissue wet weight respectively) and that the photoreceptor cells of the ventral and lateral eye preparations are a mixture of octopamine and tyramine metabolites. There is no detectable accumulation of octopamine metabolite when tyramine is used as the precursor.
CIRCADIAN RHYTHM IN THE EYE OF APLYSIA RECORDED IN VIVO.
G. D. Bloch, Department of Biology, University of Virginia, Charlottesville, Va. 22903.

The eye of Aplysia expresses a circadian rhythm (CR) in the frequency of compound action potentials (CAPs) when recorded in vitro (Jacklet, Science 164:562-563, 1969). Aplysia also exhibits a circadian rhythm in the frequency of its spontaneous activity. Although extracellular photoreceptors and oscillators are involved in controlling the locomotor rhythm, changes in locomotor pattern following eye removal suggest that the eye also contributes to locomotor control via an ocular circadian oscillator (Strumwasser, Physiologist 16:9-42, 1973; Lickley et al., J. Comp. Physiol. 118:121-143, 1977). Unfortunately, the expression and waveform of an ocular CR in vivo cannot be confidently inferred from in vitro data. The eye of the cephalopod contains different fibers which, when active, modify the patterning and frequency of CAPs (Esken, Z. Vgl. Physiol. 74:353-361, 1975). Thus to further define the role of the ocular CR in controlling locomotor activity it is necessary to: (1) confirm that a CR in activity is expressed in vivo and (2) evaluate the in vivo phase relationship between the ocular and locomotor CRs.

In situ optic nerve activity was recorded by means of 0.03 " teflon coated Pt-ir electrodes. Continuous recordings were made for up to 90 hr from Aplysia maintained in 21 glass aquaria. CAPs recorded in this manner displayed amplitudes ranging from 20-100 μV and in most cases showed irregular burst patterns, suggesting ineffective electrode coupling. The results indicate the presence of an ocular CR in vivo. When Aplysia were maintained in constant darkness CAP frequency peaks (200-400/hr) occurred at projected dawn with near peak levels persisting for 4-5 hr. The waveform of the CAPs in Aplysia was similar to CRs obtained in vitro with the cerebral ganglion left attached. Aplysia were placed on light cycles (LD 12:12), CAP frequencies remained near peak levels for the entire photoperiod, but decreased to low levels at dusk (5-10/hr) remaining at this baseline until 2-3 hr before dawn. Simultaneous recording of locomotor activity by time-lapse cinematography indicated that locomotor onset closely followed the predawn increase in CAP activity.

Supported by NIH 52894.

VASCULARIZATION OF THE ABOLITIONAL CENTRAL NERVOUS SYSTEM OF THE CRAYFISH: CONSEQUENCES FOR PHYSIOLOGY.
Stephen R. Brown*, John H. Byrne, Department of Biological Sciences, Cornell University, New York, NY 14853.

In the process of preparing a paper for a meeting of the Association for Research on Behavior Disorders, I was led to consider the matter of vascularization of the cephalopod nervous system. Crayfish offer a simple test system in which these questions can be examined. An important difference between crayfish and cephalopods is that the former have a closed and the latter an open vascular system. Both of these systems can be examined by means of dye injection or by perfusion with high sensitivity and accuracy. The vascularization of the crayfish CNS has been studied in detail by Koster and his associates (Koster, Exp. Cell Res. 14:439-450, 1958; Koster & Schuurman, Exp. Cell Res. 16:438-450, 1959). The present study is an extension of these investigations, with particular emphasis on the cerebral ganglion. The results indicate the presence of an ocular CR in vivo. When Aplysia were maintained in constant darkness CAP frequency peaks (200-400/hr) occurred at projected dawn with near peak levels persisting for 4-5 hr. The waveform of the CAPs in Aplysia was similar to CRs obtained in vitro with the cerebral ganglion left attached. Aplysia were placed on light cycles (LD 12:12), CAP frequencies remained near peak levels for the entire photoperiod, but decreased to low levels at dusk (5-10/hr) remaining at this baseline until 2-3 hr before dawn. Simultaneous recording of locomotor activity by time-lapse cinematography indicated that locomotor onset closely followed the predawn increase in CAP activity.

Supported by NIH 52894.

A COMPARISON OF THE NEURAL CIRCUITS FOR INKING BEHAVIOR AND GILL-WITHDRAWAL IN APLYSIA CALIFORNICA.
John H. Byrne, Department of Physiology, School of Medicine, University of Pittsburgh, PA 15260.

As our understanding of the neural control of simple behaviors progresses, it becomes of interest to examine to what extent different behaviors utilize different neural substrates. Defensive inking behavior and gill-withdrawal in Aplysia offer simple test systems in which these questions can be examined. Aplysia has been a good model system for both of these behaviors, with the CRs for inking being well characterized by Castellucci et al. (in prep) and the CR for gill-withdrawal by Castellucci et al. (in prep) and the CR for gill-withdrawal by Castellucci et al. (in prep) and the CR for gill-withdrawal by Castellucci et al. (in prep) and the CR for gill-withdrawal by Castellucci et al. (in prep). The results indicate the presence of an ocular CR in vivo. When Aplysia were maintained in constant darkness CAP frequency peaks (200-400/hr) occurred at projected dawn with near peak levels persisting for 4-5 hr. The waveform of the CAPs in Aplysia was similar to CRs obtained in vitro with the cerebral ganglion left attached. Aplysia were placed on light cycles (LD 12:12), CAP frequencies remained near peak levels for the entire photoperiod, but decreased to low levels at dusk (5-10/hr) remaining at this baseline until 2-3 hr before dawn. Simultaneous recording of locomotor activity by time-lapse cinematography indicated that locomotor onset closely followed the predawn increase in CAP activity.

Supported by NIH grants NS09457, and NS14966 and R01 0642.


One criterion that might be used in the selection of a preparation for studying the neural basis of behavior is the number and size of neurons in the central ganglia. Ganglia from three gastropod molluscs were dissected out, pinned out in a bath of a saline which was the same one used for experiments in sea water, dehydrated in acetone, cleared in methyl salicylate and mounted between coverslips. Cell counts were performed with the aid of a compound microscope equipped with Nomarski differential interference contrast optics. The saline used for counting was 0.8 M sucrose, 10 μM MgCl2, 5 μM CaCl2. Nine preparations were made from three species: 3 Navanax, 3 Aplysia and 3 Aplysia californica. Counts were made on the gill, the cerebral and the pleural ganglia. The results indicate the presence of an ocular CR in vivo. When Aplysia were maintained in constant darkness CAP frequency peaks (200-400/hr) occurred at projected dawn with near peak levels persisting for 4-5 hr. The waveform of the CAPs in Aplysia was similar to CRs obtained in vitro with the cerebral ganglion left attached. Aplysia were placed on light cycles (LD 12:12), CAP frequencies remained near peak levels for the entire photoperiod, but decreased to low levels at dusk (5-10/hr) remaining at this baseline until 2-3 hr before dawn. Simultaneous recording of locomotor activity by time-lapse cinematography indicated that locomotor onset closely followed the predawn increase in CAP activity.

Supported by NIH grants NS09457, and NS14966 and R01 0642.

Previous work has identified two populations of neurons in the brain ganglia that control pharyngeal expansion: expansion neurons innervate radial fibers, causing pharyngeal expansion and circumferential neurons innervate circumferential fibers, causing pharyngeal contraction. The present study provides additional evidence that both neuronal types make monosynaptic connections onto muscle and shows that identified cells innervate specific regions on the pharynx. Two populations of circumferential motorneurons (MN) were described, each innervating distinct bands which are remarkably constant in number over a 15-fold range of pharyngeal weights for both dorsolateral and ventral regions. The dorsolateral MNs innervate circumferential #25 to 31 via the dorsal pharyngeal nerves. A smaller left ventral cell innervates the posterior area of muscle via specific combinations of the dorsal and ventral pharyngeal nerves on each side. Identified circumferential and appropriate antidromic latencies with peripheral electrical stimulation. MNs were recorded from a grid work of up to 40 pin electrodes passed through the pharyngeal wall at specific bands to determine motorneuron (MN) fields. Identified circumferential cells appear to innervate single subpopulations of bands. The anterior pharynx was divided into a distinct subgroup. Four MNs innervate this phasone: one on each side activates it bilaterally, another on each side activates it ipsilaterally. Several additional MNs innervate anterior and posterior bands. Expansion MNs innervate particular areas of radial muscle via specific combinations of the dorsal and ventral pharyngeal nerve. Both giant (G) cells innervate the entire pharyngeal nerve, while smaller nerves innervated by EMG recordings and serial section reconstruction. It is not yet determined whether each G cell innervates every radial fiber. The left ventral medium sized (M) cell innervates the anterior dorsal pharynx from circumferential #25 to 31 via the dorsal pharyngeal nerves. Another smaller left ventral MN innervates the posterior area of the ipsilateral pharynx via ipsilateral dorsal and ventral pharyngeal nerve. Other cells with various motor field sizes have been observed but not adequately characterized with respect to identifiability and uniformity. This work suggests a framework for studying the contribution of individual MNs to generation of feeding behavior. Supported (in part) by NIH grant 5T32GM7288.


When the bag cell (BC) neurons of A. californica are electrically stimulated after an 18 hr starvation period, expansion neurons innervate radial fibers, causing pharyngeal expansion and circumferential neurons innervate circumferential fibers, causing pharyngeal contraction. The present study provides additional evidence that both neuronal types make monosynaptic connections onto muscle and shows that identified cells innervate specific regions on the pharynx. Two populations of circumferential motorneurons (MN) were described, each innervating distinct bands which are remarkably constant in number over a 15-fold range of pharyngeal weights for both dorsolateral and ventral regions. The dorsolateral MNs innervate circumferential #25 to 31 via the dorsal pharyngeal nerves. A smaller left ventral cell innervates the posterior area of muscle via specific combinations of the dorsal and ventral pharyngeal nerves on each side. Identified circumferential and appropriate antidromic latencies with peripheral electrical stimulation. MNs were recorded from a grid work of up to 40 pin electrodes passed through the pharyngeal wall at specific bands to determine motorneuron (MN) fields. Identified circumferential cells appear to innervate single subpopulations of bands. The anterior pharynx was divided into a distinct subgroup. Four MNs innervate this phasone: one on each side activates it bilaterally, another on each side activates it ipsilaterally. Several additional MNs innervate anterior and posterior bands. Expansion MNs innervate particular areas of radial muscle via specific combinations of the dorsal and ventral pharyngeal nerve. Both giant (G) cells innervate the entire pharyngeal nerve, while smaller nerves innervated by EMG recordings and serial section reconstruction. It is not yet determined whether each G cell innervates every radial fiber. The left ventral medium sized (M) cell innervates the anterior dorsal pharynx from circumferential #25 to 31 via the dorsal pharyngeal nerves. Another smaller left ventral MN innervates the posterior area of the ipsilateral pharynx via ipsilateral dorsal and ventral pharyngeal nerve. Other cells with various motor field sizes have been observed but not adequately characterized with respect to identifiability and uniformity. This work suggests a framework for studying the contribution of individual MNs to generation of feeding behavior. Supported (in part) by NIH grant 5T32GM7288.

Laboratory-reared animals may show reduced variability in behavioral and electrophysiological studies. Recently, laboratory rearing of the sea slug Aplysia californica results in a behavioral plasticity not seen in field-reared animals. Hermisenda exhibit photopositive behavior when maintained on a 6.5 hour light: 17.5 hour dark or 12 hour light: 12 hour dark schedule and rearing the light:dark cycle approximately equal to the response to light was assessed by monitoring latencies of individual Hermisenda to enter an illuminated area. After entering the light field Hermisenda remain in the illuminated area significantly greater time of time during the observation periods as compared with animals tested in darkness (p < 0.01). Response latencies to move from the light after 15 sec to the dark are significantly longer following testing for approximately 3 days and these results imply an induced transmembrane increase in the number of statoconia per statocyst. Dissection of animals from the F3 generation revealed variation in the number of statoconia per statocyst. The morphological differences may help elucidate the role of the statocyst in the behavioral modification and the reduced behavioral variability of laboratory-reared Hermisenda following training.

BRITISH BIOCHEMICAL SOCIETY MEETING ON INVERTEBRATE NEUROBIOLOGY 792

1-I CHANGES IN RESPONSE TO CYCLIC NUCLEOTIDE AGENTS IN APLYSIA NEURONS. Peter L. F. Drake and Steven N. Treistman, Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The bursting cells R15 in Aplysia and F-I in Helix are subject to synaptic and hormonal alteration which persists for long periods and may be mediated by cyclic nucleotides (Treistman and Levitan, Nature, 1976; PMAS, 1976). We have shown (Brain Research, 1977) that agents which affect cyclic nucleotide levels (e.g., phosphodiesterase inhibitors, CAMP derivatives, and adenylate cyclase activators) elicit responses in many Aplysia neurons other than R15, and these responses include the induction of bursting in motoneurons. When we clamp the I-V characteristic in one such cell, the metacephalcal cell (MC), which bursts after incubation with the phosphodiesterase inhibitor IBMX, shows the induction of anomalous rectification and hysteresis in response to triangular ramp current injection.

We measured phase-response curves by presenting stimulus puffs at various parts of the cycle. Stimulation late in the cycle can phase advance the INT II oscillator by triggering a short-latency INT II burst. However, if presented early (up to 15 sec after a spontaneous burst), the stimulus does not trigger an INT II burst. We also examined entrainment of the INT II oscillator by varying the phase of the stimulus. The probability of triggering INT II bursts is dependent on INT II within a particular range which is close to the free run period. Entrainment tends to break down at lower INT II.

The behavioral significance of the INT II burst can be demonstrated by video analysis of gill contractions in restrained animals. The INT II burst contributed to the standard wind puffs which produces a cough-like response in the gill machine. The INT II burst can be triggered by the application of a standard wind puff which produces a cough-like response in the gill machine. The INT II burst can be triggered by the application of a standard wind puff which produces a cough-like response in the gill machine. The INT II burst can be triggered by the application of a standard wind puff which produces a cough-like response in the gill machine.
ANOMALOUS CONNECTIONS BETWEEN IDENTIFIED NEURONS FOLLOWING MICRO-LESION IN THE LARVAL PHARMA MICROSCAPHUS BORENBERGENI. David R. Friedlander* and Cyrus Levinthal. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

The specificity in the formation of synapses between identified neurons was investigated by following the anatomical effects of killing the cell body of a giant motorneurone (GNN) in the developing giant fiber system responsible for the escape response in twospotted crayfish. Analysis was done by observation of complete serial thick (1 µm) and thin (0.1 µm) sections of ganglia, fixed from 1 to 56 days after being irradiated in the first larval stage. The giant fiber system in the adult prawn consists of two pairs of bilaterally symmetric giant interneurons (GIs) and the GNMs, a pair of fused neurons, whose central process splits in two axes that leave the CNS through the motor roots, separately innervating left and right flexor muscles. Each GI synapse exclusively with the ipsilateral GNN axon in the motor root region by means of a short collateral. Holmes, W., Philos. Trans. B 231:293-311, 1942; Macagno, E. and Friedlander, D.R., Soc. Neurosci. Abst. 2:174, 1976). The adult GNN synctium is the result of the fusion of individual larval neurons, with axons contralateral to their somata. Connections between the GIs and the GNMs are already established at the time of lesion, the first day after hatching.

Microlesions are produced by focusing a beam of light which has a significant percentage of its energy in the ultraviolet, on a region of the ganglion containing the GNM, immediately above the thin ventral capsule. Within a day, the GNM soma cannot be recognized. Signs of axonal degeneration have been observed two days post-lesion; complete disappearance of the axon takes about 10 days after the light microlesion. The remarkable ability of some invertebrate neurons to survive without a soma (Hoy, R.P., J. Exp. Zool. 172:219-232, 1969) is not present in early larval stages of the crayfish.

The most common result of the removal of the GNM axon is the resorption of the collaterals of the GIs deprived of their target. More interestingly, in 30% of the ganglia analyzed 21 or more days after irradiation, one or more anomalous connections were found, usually involving a GII deprived of its normal target, with the GII synapsing on the remaining two GIs. This synaptic plasticity supports the notion of a hierarchical order in the rules governing the formation of neuronal connections, where neuron type ranks higher than laterality.

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The locomotor circadian activity was investigated in the intact crayfish under conditions of complete darkness and constant temperature. In order to obtain information about the sensitivity of the circadian system, among several possible experimental designs we chose one that involves only administration of a light stimulus to the organism under "free running" conditions. The light application was done along the twenty-four hours in different "circadian time". The results of a series of experiments are phase advances or delays which have been summarized in the so-called "phase-response curve".

We observed that one complete oscillation of the phase response curve occurred during each circadian oscillation and that there is only one position of the cycle during which the stimulus produces phase advances and each single one during which phase delay results. This unimodal phase response curve which exhibit different oscillators is essential for adaptive capability of "biological clocks" to periodical environmental changes.

SYNAPTOSIS IN THE FLY'S VISUAL SYSTEM. A. Förödenb* and I.A. Meinershagen, Life Sciences Centre, Dalhousie University, Halifax, N.S. Canada.

The formation and selection of synapses during development is the last step in a chain of cellular control mechanisms by which neurons interconnect in the central nervous system. To study the specificity of the rules which control this step we have examined anatomically populations of synapses in the first optic neuropil, or lamina, of the fly Musca domestica adult and in the immature stages. Synapses have been scored from limited series of up to 50 consecutive micrographs prepared from material fixed and processed conventionally for electron microscopy. Synapses are scored in classes according to the identity of the presynaptic element and for each representative the identity of up to four postsynaptic processes were established. Studies of synapses from serial sections provides not only a direct count of synapse frequency but also some measure of the variability with which different postsynaptic elements associate at a given synapse class, addressing the question of the specificity with which synaptic formation occurs. Analysis of different developmental stages provides evidence on how this specificity is attained and may be correlated with the acquisition of adult synaptic ultrastructure.

We have found that synapses form with great specificity. In the adult, the first-order synapse is an elongate tetrad with dyadic symmetry. > 97% of all examples are consistent with LI and II neurons which have lateral terminal positions as previously described (Burkhardt and Strausfeld: Cell and Tiss. Res. 173, 287) while there is variability in the occupants of the polar dendritic positions. The orientation of synapse long axis is also random. The synapses which have the remarkable ability of some invertebrate neurons to survive without a soma (Hoy, R.P., J. Exp. Zool. 172:219-232, 1969) is not present in early larval stages of the crayfish.

DYE COUPLING INDICATES THE STRUCTURE OF ELECTROTROCHICALLY COUPLED NETWORKS IN SENSORY NEUROPILOT OF THE CRAYFISH BRAIN. Raymon M. Glantz, Mark Kirk, and Howard Wood. Dept. Biol., Rice University, Houston, TX 77001.

Interneurons which arise in the visual and antennal neuropil of the crayfish brain descend to the more caudal ganglia via the circumesophageal connectives. These neurons are excited by stimuli to the sensory organs of the head and control or modify the activity of lower motor centers in thoracic and abdominal ganglia. Recent studies have shown that within each neuropil there is an active recruitment of receptors by afferent stimulation of different features and receptive field (Glantz, R.N., J. Neurophysiol. 41:1297-1313, 1314-1327, 1978). Several lines of evidence indicates that the formation of synaptic connections is controlled by two factors: (a) The synaptic delay is 0.1 ms or less; (b) The synaptic potentials follow presynaptic spike trains at rates of up to 2000 Hz without after synaptic transmission. (c) The two synaptic terminals are polarizing and hyperpolarizing injected currents. Morphological analysis of the ensemble interactions has been carried out with the fluorescent dye Lucifer Yellow CH supplied by W.H. Stewart of the N.I.H. Stewart (Cell, 14:741-759, 1978) has summarized evidence that dye coupling is diagnostic for electrotonic coupling. Injection of single antennal interneurons has resulted in the dye spreading up to three additional cells. Transynaptic dye migration is observed in about 60% of the injected preparations. The soma of dye coupled cells may be adjacent to each other or separated by the width of the fiber. The axons of the columnar cells frequently exit in each of the two circumesophageal connectives. Individual cells can pass up to three distinct dendritic zones spanning across the entire width and length of the neuropil. The dendrites are punctuated with multiple varicosities suggestive of chemical synaptogenic endings. Central neurites of up to 25μm in diameter are infrequently observed.

The reciprocal coupling promotes strong synchronization in the discharge of the parallel descending axons. Since the individual fibers are generally subthreshold, the reciprocal synchronization of a given pair of neurons depends upon the coactivation of other elements in the network. Different stimulus conditions control the different central synapses. The stimulus conditions identify both the activated subpopulation and the spatial pattern of coordination within the active subpopulation (Wood, H. Ph.D. thesis, Rice Univ., 1978).
TRANSFER OF HABITUATION FROM ONE PATHWAY TO ANOTHER IN APLYSIA. J. Goldberg* and K. Lukowiak* (SPOM: W. L. Veale). Div. Med. Physiol., Fac. of Med., Univ. of Calgary, Calgary, ALTA T2N 1R4

The gill withdrawal reflex and its subsequent habituation in Aplysia can be evoked by repeated stimulation of two distinct stimuli. Tactile stimulation of either the siphon evokes these gill behaviors. It has been suggested that the neural pathway which mediates these gill behaviors evoked by siphon stimulation is biologically different from that of the other pathway which mediates the reflex behavior evoked by gill stimulation. We found that habituation of the reflex evoked in one pathway generalizes is transferred to the evoked reflex in the other pathway. Thus, the results indicate that an interaction does exist between the two pathways. This supports the idea that the neural circuitry mediating gill withdrawal behaviors involves an integrated system. The following experiments were done:

1) Tactile stimuli (1 g) were presented to the gill once every 20 minutes and the reflex did not habituate. 2) If the siphon was stimulated (1 g) 10 times (ISI=30s) (which resulted in habituation of the reflex) just before a stimulus was presented to the gill, it was found that the amplitude of the reflex evoked by the gill stimulation was significantly smaller than the control. 3) It was further found that the rate of habituation evoked by repeated gill stimulation was faster following the interposition of siphon stimuli than in control habituation sessions. 4) Transfer of habituation was also apparent at the neuronal level. The number of action potentials evoked in gill motor neuron L7 by a single tactile stimulation of the gill was reduced following a siphon stimulation habituation run. 5) Similar results were obtained when the gill stimuli and siphon stimuli were reversed. 6) A central nervous system (abdominal ganglion) is not necessary for the transfer of habituation to occur, since similar results were obtained in experiments where the abdominal ganglion was removed. 7) These data show that there are at least 2 sites where the transfer of habituation can occur; centrally at the sensory-gill motor neuron synapse, and peripherally in the gill.

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Studies of the physiology of the bursting process of cells L2 - L6 and R15 of Aplysia californica have shown that the oscillatory currents responsible for burst firing are endogenous to the cell, but that synaptic input can modify the bursting pattern. Recently, it was demonstrated that the inhibition of bursting by acetylcholine in L2 - L6 and by dopamine (DA) in R15 was due to the abolition of the negative resistance region in the current-voltage (I-V) relationship of these neurons.

We found that the threshold DA dose for an effect on the I-V relationship was 5 × 10⁻⁴ M. The effects of DA at higher concentrations on the I-V relationship were studied under voltage clamp conditions using the single micro-electrode technique. The threshold DA dose for an effect on the I-V relationship is 10⁻⁴ M. With increasing doses, the negative resistance region of the curve is progressively reduced. Doses of 6 X 10⁻⁴ M DA tend to decrease and abolish bursting activity. Washing the ganglion with sea water restores the I-V relationship to the control conditions. Addition of imipramine or pargyline to the perfusate in doses shown to affect DA metabolism in molluscan systems do not change the characteristics of the dose-response relationship. Experiments using putative dopaminergic agonists and antagonists to characterize the receptor mediating this response will be discussed.

AXONAL SPROUTING IN AN IDENTIFIABLE LARVAL LOBSTER MOTO NEURON. C.K. Govind and Joanne Pearce*. Scarborough Coll., Univ. of Toronto, Wexwell Hill, Ontario, M1C 1A4, Canada.

Axonal sprouting is a well known but poorly understood phenomenon of the nervous system which should ideally be examined in a single identifiable neuron. The excitor motoneuron to the limb receptor muscle in lobster, Homarus americanus, provides this opportunity when examined in the 1st larval stage when it sprouts vigorously to innervate the rapidly growing muscle. Consequently almost two thirds (60 μm) of the entire length of the motoneuron was examined by serial section electron microscopy. In the 1st larval stage there is little or no inhibitory innervation. The excitor axon however, traverses the width of the muscle and sprouts branches toward the tendon and exoskeleton ends. This primary sprouting occurs in regions of the axon which differentiate into terminals by contacting preexisting granular tissue and accumulating agranular synaptic vesicles. The terminals possess presynaptic dense bars which are regarded as active sites of transmitter release. These secretions are released whether by themselves or as part of fully formed synapses. The newly formed sprout is in contact with muscle sarcoplasm, is filled with synaptic vesicles and has synapses; in effect it is a terminal. The primary sprout gives rise to secondary sprouts which have similar characteristics. The pattern of innervation is variable, with some axons not innervating the muscle at all. Secondary sprouting is more common. Consequently the target tissue (muscle) may induce sprouting which would be a mechanism for regulating the innervation of the muscle during development and growth.

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The heartbeat in spiders is not a single response but a sequential chain of events. The heartbeat is initiated and coordinated by a thread-like cardiac ganglion situated along the dorsal mid-line of the heart. The ganglion generates the cardiac rhythm. Injections of DA or other pharmacologic agents were selectively added to the ganglion, to determine whether the cardiac rhythm is influenced by these agents. The threshold DA dose for an effect on the I-V relationship was 10⁻⁴ M. The effects of DA at higher concentrations were studied under voltage clamp conditions using the single micro-electrode technique. The threshold DA dose for an effect on the I-V relationship was 10⁻⁴ M. With increasing doses, the negative resistance region in the current-voltage (I-V) relationship was progressively reduced. Doses of 5 × 10⁻⁴ M tend to linearize the curve and effectively abolish bursting activity. Washing the ganglion with sea water restores the I-V relationship to the control conditions. The frequency of the cardiac bursts and their duration were altered accordingly. Therefore, both cardio-inhibition and cardioacceleration can be achieved by activity in these nerves.

There appears to be only one excitatory and one inhibitory axon in each cardioaccelerative nerve. Only one voltage dependent channel of each type can be found. A conclusion is that there are two excitatory and two inhibitory neurons that arise from opposite sides of the main abdominal ventral nerve and gives rise to three branches one of which is VIIIb. Nerve VIIIb further branches and each such bundle is the cardio­regulatory nerve. Consequently, there is a pair of cardio­regulatory nerves, each innervating the cardiac ganglion at about the same level, but from opposite sides.

Conclusive demonstration that VIIIb contains cardioaccelerative neurons was obtained by stimulating it electrically while simultaneously recording the activity of the cardiac ganglion and the contractions of the heart. Both increases and decreases in the rate and amplitude of the beat could be evoked under appropriate stimulation conditions. The frequency of the cardiac bursts and their duration were altered accordingly. Therefore, both cardio-inhibition and cardioacceleration can be achieved by activity in these nerves.

The former are more spherical than the latter. This permits identification of the excitatory motoneuron in VIIIb extending to the cardiac ganglion just anterior to the first pair of ostia. Nerve VIII is paired; each member of the pair arises from opposite sides of the main abdominal ventral nerve and gives rise to three branches one of which is VIIIb. Nerve VIIIb further branches and each such bundle is the cardio­regulatory nerve. Consequently, there is a pair of cardio­regulatory nerves, each innervating the cardiac ganglion at about the same level, but from opposite sides.

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DCS is a McKnight Scholar in Neuroscience.

There are however nearby specializations between primary branches synapses may mediate uncoupling of expansion motoneurons. (Granzow and Kater, Neuroscience 2:1049-1063, 1977.)

These cerebral neurons of Helisoma have characteristics in common with the Metacerebral Cells of other gastropods including the context of serotonin, the axonal diameter with branches extending into buccal ganglia nerve trunks, and their ability to influence buccal ganglia neurons. These commonalities indicate that cerebral cells of Helisoma might be homologous to the Metacerebral Cells. However, we have found certain physiological properties of Helisoma cerebral cells which differ from those of the Metacerebral Cells of Helis and Aplysia. These properties include, among others, a high spontaneous firing rate (approx. 2 spikes/sec), a predominant of tonic inhibitory synaptic potentials, and a linear I-V relationship, i.e., no anomalous rectification of the membrane. The behavioral significance of these differences has not yet been determined. However, the cerebral cells of Helisoma strongly affect the pattern of the buccal ganglia feeding motor output, whereas the effect of activity in the Metacerebral Cells of Aplysia is most apparent as a potentiation of intensity of motoneuron activity. Since the serotonergic cerebral cells may have somewhat different roles in various gastropods, it is not surprising to find differences related to the integration processes of these cells.

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A serial reconstruction of 3u sections through the buccal ganglia of Nayanax inermis has revealed a group of large axons (10-50u diameter) in a bundle which runs mediolaterally through both ganglia in a linear fashion and crosses the midline commissure. Most or all of these large axons belong to physiologically identified motoneurons innervating radial muscle, which are responsible for pharyngeal expansion. Some of this group of motoneurons were not previously recognized as motoneurons that relay information to the buccal ganglia. Presently, there appear to be six to eight identifiable expansion motoneurons in each buccal ganglion innervating partially overlapping or discrete regions of the buccal mass. These motoneurons have been identified morphologically in the reconstructed series by comparison to a catalogue of physiologically identified expansion motoneurons that had been injected with the dye Lucifer Yellow CH. The morphological criteria for identification include the size, shape and position of the soma and the number, size and distribution of the neurites, e.g. do axons exit only ipsilaterally or both ipsilaterally and contralaterally.

The expansion motoneurons are known to be electrophysiologically connected to one another by the largest of the large motoneurons, the G (cerebral cells). This coupling can be blocked by synaptic input from a population of inhibitory neurons at least some of which are also serotonergic. The coupling and uncoupling of chemical synapses may be localized within or very near the bundle of large axons. Intracellular recordings show stronger electrotonic coupling from soma to postsynaptic axon than from soma to postsynaptic soma. Also, in vivo recordings in the G cell are larger with faster rise times in the axon than in soma. Examination of the axon bundle in thin section reveals a heavy glial contact between axons. There are however nearby specializations between primary branches of the axons which resemble gap junctions in some respects and extracellular lanthanum treatment is being used to further characterize these appositions. Chemical synapses are also found on the axons and their primary branches in this region. These synapses may mediate uncoupling of motoneurons.

Supported by NIH Grants NS 13881 to C.M.H. and NS 12828 and RR 05763 to G.R.S. L.H.M. is a McKnight Scholar in Neuroscience.


The voltage-sensitive sodium channel of Drosophila larval motor nerves has been studied using two neurotoxins, tetrodotoxin and saxitoxin, which are known to bind specifically to sodium channels and block the sodium channel. Since the voltage-sensitive sodium channel is part of the voltage-sensitive sodium channel. Binding and electrophysiological studies were done on a mutant, itama, which is abnormal in sensory. This mutation does not appear to affect the density or structure of sodium channels since there is no difference between mutant and wild-type with regard to physiological sensitivity to tetrodotoxin and [3H]saxitoxin binding parameters. These studies provide techniques which can be used to identify mutants with defects in the sodium channel.

Supported by NIH Grants NS 13881 to L.H.M. and NS 12828 and RR 05763 to G.R.S. L.H.M. is a McKnight Scholar in Neuroscience.
ADDITIONS TO THE PHYSIOLOGY OF THE STOMATOGASTRIC GANGLION (STG) OF THE SPINY LOBSTER, PANULIRUS interruptus: D. R. Martine, D. Y. Casale, C. D. Schlichting, B. Breyder Lab., New Milford, New Milford, NJ. We present additional properties and connections of specific cells in isolated STG to aid understanding pattern generation by a simple network (architectures confocal in cooperation with D. Russell ("m") or K. Graubard and J. Raper ("r")).

1. **A** Neuron compared to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.

2. **LP** Neuron has a rectifying electronic connection to PEs.†

3. **FT** Neurons are of two types, PEs ("early") and PLs ("late") during PD burst and terminate during PD repolarization, likely due to its electrical coupling to PD. Addition of AB can lead to a new gap in PD pattern, with two PD bursts per cycle. Transmitter release from IP/AB via electroeless paths may explain weak delayed PD IPSP's on LP, P1, and O, blocked by hyperpolarizing PD.

4. **py** Neurons are of two types: PEs ("early") and PLs ("late") during PD burst and terminate during PD repolarization, likely due to its electrical coupling to PD. Addition of AB can lead to a new gap in PD pattern, with two PD bursts per cycle. Transmitter release from IP/AB via electrophysiological pathways may explain weak delayed PD IPSP's on LP, P1, and O, blocked by hyperpolarizing PD.

5. **Pe** Neurons produce a phase delay of CP. In PYs this is associated with a rapidly developing slowly inactivating IPSP on LP and IC. This produces a slowly developing repetitive firing as compared to other cells (LP, VD, D). It appears to be a primary receptor making weak electrical connections to LP. A similar result has been obtained when LP was stimulated electrically during PD burst and terminated during PD repolarization, likely due to its electrical coupling to PD. Addition of AB can lead to a new gap in PD pattern, with two PD bursts per cycle. Transmitter release from IP/AB via electroeless paths may explain weak delayed PD IPSP's on LP, P1, and O, blocked by hyperpolarizing PD.

6. **E** Neuron appears to receive major inhibition from AB and 1. **A** Neuron compared to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.

7. **A** Neuron compared to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.

8. **PD** Neuron has a rectifying electronic connection to PEs.†

9. **PL** Neuron appears to receive major inhibition from AB and 1. **A** Neuron compared to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.

10. **a** effect from AB to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.

11. **B** Neuron appears to receive major inhibition from AB and 1. **A** Neuron compared to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.

12. **A** Neuron compared to PEs (intracellular recordings): a) often has twice as many spikes in each summed evoked EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to depolarization.
Distinguished these pairs of fibers by using both physiological and structural criteria.

We investigated the effects of proprioceptive feedback on the period of the motor pattern by interfering with the movements of individual swimmerets. Experiments were performed on Procambarus clarkii (Girard) and Panulirus longicarpus. The abdomen was separated from the body and pinned in a Sylgard lined dish which allowed the swimmerets to move freely. Activity of the motor neurons was monitored with extracellular pin electrodes. Bundles of axons containing command fibers were stripped from the ventral nerve cord anterior to the second ganglion and stimulated with suction electrodes. When a command fiber was located and a regular motor pattern produced, we interfered with one swimmeret's movement with a pin held in its path. The swimmeret was held in the fully retracted position which occurs at the end of a power stroke for several bursts then allowed to move freely. We observed four different kinds of results. Either the period ceased, did not change, increased, or bursting was inhibited. We think these differences reflect the individual command fiber isolated for stimulation.

A long lasting afterdischarge can be generated in the neuropeptidergic bag cells of the abdominal ganglion of Aplysia following brief electrical stimulation for several hours. After 16-24 hr (Day 2) full recovery takes place (MD for Day 1  = 30.2 min, N=16; MD for Day 2 = 4.0 min, N=5). Previous work has shown that bag cell axons are insensitive to 8-benzylthio cAMP. These treatments resulted in 51% (N=8) greater incorporation into TCA-insoluble material from the bag cell somata and surrounding connective tissue, which contains many bag cell neurites. Increased phosphorylation into one protein band was observed with either electrical stimulation or treatment with cAMP analogue when compared with controls. Microdensitometric analysis of the gel autoradiograms increased a 41-kilodalton (41-kDa) protein phosphorylation of this protein. The m.w. of this phosphoprotein was estimated to be 22,000 daltons by its migration on SDS polyacrylamide gels. The phosphorylation of the 22,000 m.w. protein in the bag cell region occurs within 5 minutes of the onset of afterdischarge. We have also observed phosphorylation of two high m.w. (approx. 120,000 and 125,000 dalton) proteins in the bag cell region. The long time course of the excitatory changes observed in this system (30 minutes) may allow correlation of the time course of protein phosphorylation with the onset and termination of the afterdischarge. (K.R.J. is a Gordon Ross Medical Research Foundation fellow.)

Supported by NSF grant BNS 78-10516.
CHARACTERISTICS OF AND INTERACTIONS BETWEEN SEPARATE BRANCH SPIKES FOUND IN CRAYFISH SENSORY INTERNEURONS. Mark Kirk and Raymon Grazsi. Dept. Biol., Rice University, Houston, TX 77001.

Intracellular recording from neuropil processes of the crayfish brain and abdominal ganglia reveal action potentials which can be distinguished as spikes originating from separate branches of the same cell. Each branch spike is usually seen to have a distinct threshold to injected currents. They may occur in a variety of temporal sequences with respect to each other. Often EPSPs precede either event when they occur separately or alternatively one branch spike may lead into the other. In special cases, such as during a step of depolarizing current, one spike is seen to entrain the other's discharge. The branch spikes may have overlapping or completely separate sensory receptive fields, and in the extreme case may subtend different sensory modalities. During recordings from neurons of the supraesophageal ganglion, in which the branch spike corresponding to the cell's descending "main" axon discharge is usually identified, it can be seen that summation of the "dendritic" spike with other depolarizing events (injected outward current, PSPs) is required for the main axon to fire. In some instances the occurrence of the axon spike is invariably associated with the sequential activation of the "dendritic" spike on the descending phase of the axon spike. Conversely, several observations have been made on neurons in which the "dendritic" spike and main axon spike were completely independent. The latter observation suggests that the "dendritic" spikes may have a role in communication or integration that does not involve the axonal process at all.


To assess what effects deafferentation might have on metabolism in the TG, we compared incorporation of [3H]-leucine into TG nerve cells. Between normally reared (control) crickets and those receiving bilateral deafferentation throughout development and changes in amount of TG protein content after removal from adults. Chronic deafferentation resulted in statistically significant reduction (p < 0.05) in TG protein content when compared to control TG's. Such decreases were in accord with marked reduction in neuropil volume observed in histological sections of deafferented TG. However, when adult crickets were subjected to short-term deafferentation following development, in vitro, only a slight (p > 0.05) decrease in total TG protein was noted, probably ascribable to degeneration and loss of cefal sensory axons, terminals, and giant elements.

We then compared in vitro incorporation of [3H]-leucine into TG insoluble protein between control and chronically deafferented TG's, TCA insoluble protein between control and chronically deafferented TG's. In contrast, TG taken from crickets receiving a short-term deafferentation regimen showed no decreases in incorporation. These data indicate that chronic removal of presynaptic input to the TG does depress protein metabolism within the CNS. However, the two motor systems are reciprocally inhibitory. Plots of feeding intensity versus withdrawal stimulus intensity are not linear, but instead show several turning points and inflections. Analog electronic modeling of reciprocally inhibitory neural networks revealed increase in the intensity of withdrawal suppression with increase in the intensity of withdrawal behavior. The two motor systems are reciprocally inhibitory. Plots of feeding intensity versus withdrawal stimulus intensity are not linear, but instead show several turning points and inflections. Analog electronic modeling of reciprocally inhibitory neural networks revealed increase in the intensity of withdrawal suppression with increase in the intensity of withdrawal behavior. The two motor systems are reciprocally inhibitory.
TRANSMISSION BETWEEN CRAYFISH GIANT FIBERS AND NON-GIANT
MOTONEURONS IS NOT PRIMARILY MONOSYNAPTIC. F.B. Krasne, A.M. 

The mechanism for generation of the motor patterns that pro­duce giant fiber escape reactions in crayfish is believed to be particularly simple: The medial and lateral giant command neurons (MG/LG) are thought to distribute excitation directly to relevant fast flexor motoneurons via monosynaptic connections in each body segment, and these connections are believed to be the sole or paramount cause of motoneurons firing in appropriate extent and sequence. The direct connections of the command neurons to the motoneurons is important for at least two reasons: (1) These connections have been a source for the discovery of principles of neuronal communication such as electrical transmission at MG/LG - motor giant (moG) junctions and amplification by dendritic spikes at MG/LG - nongiant fast flexor motoneuron (FF) junctions. (2) The direct activation of motoneurons by command neurons has been viewed as an unusual form of motor system organization. Prevailing conceptual schemes picture the neuron that triggers or turns on a behavior (ie. the 'command neuron') as activating an independent pattern generating network; it is believed that command neurons may commonly bias motoneurons via monosynaptic connections but not fire them. In this view crayfish escape seems to be anomalous.

We have found that in each hemiganglion, in parallel with the monosynaptic pathway between MG/LG and the FFs, there is a disynaptic pathway in which a neuron we call the segmental driver (SD) is interposed. This disynaptic pathway is absolutely necessary and usually sufficient for driving of the FFs by MG/LG. The monosynaptic pathway between MG/LG and FFs is weak and quite incapable of firing the FFs by themselves. Supported by USPHS Grant NS0706.

In this view crayfish escape seems to be anomalous. These results, plus the ability of colchicine to mimic anatomy-induced changes in excitability (Pitman, Twedde & Cohen, Science, 178, 107 (1972)), are consistent with the hypothesis that the signal is a sudden decrease in a factor transported along the axon from muscles to the neuron soma. With regard to the nature of change in membrane excitability, we find that (1) the axotomy-induced soma spikes are sodium dependent; (2) like many other cells, FI has latent, electro­genic calcium channels that can be unmasked with tetraethyl­dihydantoin; (3) no changes in calcium conductance are de­tected following axotomy; and (4) increases in membrane excit­ability are not confined to the soma but can also be detected in the neuropil processes of FI.

The mechanism of the change is unknown, but involves a temper­ature dependent process, in which a 10°C temperature increase shifts time of onset of c. 36 hours to less than 24 hours after axotomy, and offset from c. 21 days to c. 11 days. Systemic injections of cycloheximide, a protein synthesis inhibitor, pre­vent or retard the increase in excitability if given during the period following axotomy. Cycloheximide did not affect axonally sptiking neurons. Supported by NSF Grant BNS-78-14479. J.Y.K. is an N.S.F Pre­doctoral Fellow and J. J. W. is an Alfred P. Sloan Research Fellow.

SEROTONIN-INDUCED HYPERPOLARIZATION OF APLYSIA NEURON R15 IS MEDIATED BY CYCLIC AMP. Irwin B. Levitan and Alan H. Drummond*. Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland.

Serotonin elicits alterations in the endogenous bursting activity of neuron R15 in the abdominal ganglion of Aplysia californica. Both perfusion of serotonin at concentrations greater than 0.05 μM enhances the interburst phase of the burst cycle; both the depth and the duration of the interburst hyperpolarization are increased. Higher concentrations (10-100 μM) cause complete cessation of bursting and "chemically clamp" the cell to about -70 mV. These effects are mimicked by bath application (1-500 μM) or intracellular injection of serotonin. Injection of constant depolarizing current into the Aplysia giant neuron, R2, produces a train of action potentials with an initial high firing rate followed by a gradual adaptation with slowing of the firing rate. Diphénhydantoin (DHPM) (3.8 to 10.0 x 10^-6 M) markedly slows the initial firing rate as does lanthanum (Ca²⁺-channel blocker, 1-10 M). Voltage clamp techniques were used to analyze the effect of DPH on firing rate. When the membrane potential of R2 is held at resting level (-50 to -60 mV) and then depolarized to between -35 and -45 mV for 2 seconds (sec), a changing current is seen. Initially, current is inward and peak magnitude is at­tained in 1 sec. This inward current rapidly declines to zero by 4 sec, and persists at a slowly and consistently increasing outward current is seen. On a current voltage graph of one sec. current values, the inward current is reflected by a negative slope resistance region of the curve. Both DPH and la⁴ block this inward current at concentrations which reduce early firing frequency as described above. Also, after 1 hour in calcium free 10 μM cobalt, sea water, the inward current is markedly re­duced. The blockade of inward current is reflected in the current voltage curve as disappearance of the negative slope resist­ance region and increased magnitude of outward current values. The reduction of inward current by DPH may explain the slowing of action potential generation by this drug. The similar effects of la⁴ and zero calcium with cobalt suggest that calcium may carry a portion of the inward current, and that DPH may be block­ing this calcium flux.
824 IMMUNOHISTOCHEMICAL LOCALIZATION OF A SPECIFIC SECRETORY MEMBRANE with biochemical studies, no staining of axons, neuropil or muscle or connective tissue. It was then further purified by rocket immunoelectrophoresis using an SDS membrane extract as the antigen. Other identified cells have suggested that Glycoprotein-I may be a common constituent of secretory cells. Thus, to completely understand the neural mechanisms of habituation, it is necessary to take into consideration the separation of the neural events involved in the reflex. The reflex amplitude with only the PNS intact was not significantly different than the reflex with everything intact in the controls (3.0mm ± 0.5 vs. 20.3mm ± 4.1). Again the reflex was enhanced following only Br cut (36.5mm ± 6.0). The rate of habituation with Br, Ct, Sn and PNS intact (-0.53) was significantly faster than with just PNS (-0.53). 3) The reflex with Br, Ct, Sn and PNS intact in the experimental group was significantly smaller than the reflex with everything intact in the controls (3.0mm ± 0.5 vs. 20.3mm ± 4.1). The rate of habituation with Br, Ct, Sn and PNS intact (-0.81) was significantly faster than with just PNS (-0.53). 4) The reflex with Br, Ct, Sn and PNS intact in the experimental group was significantly smaller than the reflex with everything intact in the controls (3.0mm ± 0.5 vs. 20.3mm ± 4.1). The rate of habituation with Br, Ct, Sn and PNS intact (-0.81) was significantly faster than with just PNS (-0.53).

The number of neurons was measured in specific ganglia from the nerve cord of four different species of leeches. Quantitative data were obtained by means of computer-aided techniques for the analysis of nerve structure from serially-sectioned or whole-mounted tissue (Macagno et al., 1979, Ann. Rev. Biophys. Bioeng. 8). The species studied were Hirudo medicinalis, Macrobdella decora, Haemopis marmorata (family Hirudinidae) and Haementaria ghilianii (family Glossoponiidae).

Two sets of ganglia were studied in each species: middle ganglia of Hirudo, Macrobdella and Haemopis (1 and 5), and sex ganglia (5 and 6). The middle ganglia, as well as the rest of the 21 segmental ganglia excepting 5 and 6, are thought to be quite similar. The sex ganglia are associated with the sexual organs and, visually appear to have more neurons.

The data to be presented show that: (a) the number of neurons in a specified ganglion varies by one to two percent from animal to animal of a given species; (b) the middle ganglia of a particular leech have approximately the same number of neurons, with a variation also within one or two percent; (c) the middle ganglia of Hirudo, Macrobdella and Haemopis have three to four hundred more neurons than their middle ganglia, with the exact number varying according to the species, but the sex ganglia in Haemopis are only about 20 more neurons than its middle ganglia.

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Swallowing and Regurgitation in the Isolated Nervous System of Pleurobranchaea: Distinguishing Features and Higher Order Control. Andrew D. McClellan* (SP0N: P. Sheafor). Dept. of Anatomy and Dept. of Biomed. Eng., Case Western Reserve University, Cleveland, Ohio 44106

Pleurobranchaeas regurgitation has several features in common with feeding. For example, both behaviors display rhythmic radula movement, a kinematic component commonly thought to be exclusive to feeding. Alternating buccal root activity, which underlies this rhythmic radula movement, is therefore not an adequate criterion for identification of feeding in the isolated nervous system. The motor programs for swallowing and active regurgitation can be selectively elicited in isolated nervous system preparations by extracellular stimulation of various nerves at low and high levels, respectively. Since swallowing is one of the phases of both the feeding and regurgitation responses, it presently can be behaviorally classified only in more intact preparations, where natural stimulation is possible.

In so far as feeding and regurgitation have similar motor programs, the behavioral specificity of some of the putative feeding "command" cells is in question. The metacerebral and paracerebral cells are thought to be command cells specific for feeding. Although these cells are sometimes active during the regurgitation motor pattern, particularly at its termination, they do not elicit the response when stimulated. This result suggests that these cells are non-command function during regurgitation. Several cells in the buccal ganglia do appear to specifically evoke active regurgitation. One class of cells, some of which are "ventral white cells," elicits the complete motor pattern for regurgitation when stimulated and actively bursts when the same response is evoked by nerve stimulation. A second class of cells is also capable of commanding regurgitation, although the membrane potential of these cells is only modulated during regurgitation elicited by other means.

This study implies that the higher order command cells for swallowing and regurgitation reside in the buccal ganglia, while the higher order cells for consumption feeding are located in the brain. The results also indicate that rigorous criteria must be developed for identifying feeding in isolated gastropod nervous systems; rhythmic, alternating buccal root activity does not appear sufficiently specific for feeding.

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The Myochordotonal Organ (MOO) is a muscle-sensory organ whose distal tendon spans the Neurus-Carpus joint (NC) of the crayfish claw. Its sensory cells are in series with the small Accessory Flexor muscle (AFm) and in parallel with the large working Flexor muscle (Fm) innervated by A Pe contraction and released by Fm contraction. Voluntary Flexion of the MO joint is normally produced by co-activation of Fm and A Pe. When A Pe and Fm contract simultaneously 90% of the sensory ending is proportional to the difference between the extent of contraction of Fm and A Pe. The rate sensitive MOO cells thus record the differential strain sensitivity during imposed movements of the MO joint. When the sensory cells are extended sensitive, voluntary flexion, these units gain additional proprioceptive feedback for any detectable movement of the NC joint. The MOO cells discharge during flexion, but only during flexion deceleration. Ablation of the A Pe motorneurons eliminates these responses leaving only the extension sensitivity. These additional properties are thus emergent properties of the sensory motor loop. Selective electrical stimulation of these sensory units produces one-for-one activation of the working muscle, Fm. The following sequence accompanies voluntary flexion: Co-activation of Fm and A Pe; A Pe fatigue or inadequate Pe tendon causes Pe contraction to slow down relative to A Pe contraction; MOO responds in proportion to this differential contraction; Pe receives additional input and Pe output declines as Pe rate of contraction approaches that of A Pe.

This system is a negative feedback control system in which the contractile speed of the motor program is a function of the speed of A Pe. This kind of control of intrinsic working muscle properties is important because Pe growth, injury, fatigue, heterogeneity of motor recruitment, or dampening external loads can alter limb movements arising from a stereotypic motor program. These factors are less likely to influence the contractile properties of the A Pe because of its low homogeneity of muscle fibers, size and simpler motor innervation. The MOO sensory-motor loop thus acts to reduce the complexity of Pe to some extent, insuring that successful motor programs produce similar limb movements.

(Supported by NIH NS o5243-15 and NIH 5 F32 NS 05075-02)

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HETEROGENEITY OF EXCITATORY INNERVATION ALONG SINGLE MUSCLE FIBERS IN THE LOBSTER. D.E. Meiss and C.K. Govind. Scarborough College, University of Toronto, West Hill, Ontario, Canada MIC 1A4.

Crustacean neuromuscular synapses arising from a single excitor axon are known to be well differentiated between fibers with fibers of similar numbers being known about their condition along single fibers. Focal recording techniques were used to examine the quantal transmitter release and facilitation of the synaptic terminals or synapses located at opposite ends of single muscle fibers in the singly excitatory innervated distal accessory fibers of the lobster, Homarus americanus. Synapses were reliably differentiated with respect to quantal output such that those located near the tendon end were l-15-4.12X greater than those at the opposite, exoskeletal end (p < 0.01, paired t-test). Similar differences were seen in the amount of facilitation determined from twin pulse experiments. The fine structural basis for these differences were determined by serial section electron microscopy of 10 μm segments at each end to ensure that the area of focal recording was sampled. No quantitative differences were found in the terminals or synapses in the two regions. Instead, the physiological diversity is correlated with number and size of presynaptic dense bars. Thus, the tendon end had a greater number and larger mean surface area and the area of the tendon end was 1.15-4.12X greater than that of the opposite, exoskeletal end (p < 0.01, paired t-test).

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**EXPERIMENTAL ARREST OF CLAW MUSCLE TRANSFORMATION AND ASTROPHYSICAL REVERSAL IN ALPHEID SHRIMP, DEEt. Helton and F.J. Stephens.**

In alpheid shrimp the first chelaepeda are asymmetrical and consist of a small pincer claw and an enlarged snapper claw on opposite sides of the animal. Reversal of this claw asymmetry allows loss of the snapper (Wilson, E.B., Biol. Bull., 4 (1903) 197-210). In which the pincer claw is transformed to a snapper. Transformation also occurs in response to the necessity of an otherwise intact snapper (Mellon, DeEt., F.J. Physiol., 272 (1978) 246-248). The transformation process entails profound functional and morphological changes in the original limb and requires as many as eight consecutive moults cycles to complete (Stephens, F.J., Mellon, DeEt., J. Physiol., 272 (1978) 246-248). Hill plot data gave a Hill coefficient of 0.93, indicating essentially non-cooperative binding. The Kd calculated from the Hill plot data was 5.1 × 10^-7 M, and 70-90% binding was displaced with increasing concentrations of TG in the assay mixture.

**MICROSCARIC CHOLINERGIC BINDING SITES IN TERMINAL GANGLION OF THE CRICKET CNS.**

Cholinergic binding sites have been reported to be present in the insect CNS and, in most cases, thought to be either nicotinic or mixed nicotinic-muscarinic in nature. Recently, muscarinic sites have been demonstrated in homogenates of whole Drosophila heads (J. Neurochem., 32 (1978) 136). Although electrophysiological studies indicate that acetylcholine may serve as a neurotransmitter at specific synapses in the terminal ganglion of some insects, it is unknown whether this is also true for the receptor present in this area of the CNS. We now report evidence for the presence of sites in the terminal ganglion (TG) of the cricket Acheta domestica that specifically bind the potent muscarinic antagonist 3-methylindoline benzilate (OBH).

Using a modified [3H]-QNB filter-adsorption assay, homogenates of insect TG were used to bind the ligand with high affinity. [3H]-QNB binding was displaced 70-90% by 10^-6 M scopolamine, while 10^-7 M scopolamine or prior heat treatment of the tissue reduced binding to near background levels. Incubation of TG in 10^-5 M tubocurarine and 10^-5 M xemotrine reduced binding by 40% and 85% respectively. Binding was shown to increase linearly with increasing concentrations of QNB in the assay mixture.

When increasing concentrations of labeled ligand were employed in the assays binding was observed to saturate at ca. 6 x 10^11 M [3H]-QNB. Scatchard analysis of binding curve data yielded an apparent dissociation constant (Kd) of 5.1 x 10^-7 M.

The maximum number of binding sites (Rmax) was calculated to be 3.5 x 10^7 sites per mg protein (91 fmol mg^-1 wet weight tissue). Hill plot data gave a Hill coefficient of 0.93, indicating essentially non-cooperative binding. The Kd calculated from the Hill plot data was 5.1 x 10^-7 M.

Although, at this time, cholinergic binding sites in the cricket TG have not been localized by autoradiography, physiological data generally support the notion that cholinergic neurotransmission occurs in certain neuronal pathways in the ganglion neuropile. Our findings demonstrate a rather high density of binding sites in the cricket CNS that fulfill at least some of the prerequisites for the 3H-QNB receptor (e.g. high affinity, saturability, specificity). Additional studies are now in progress to further characterize the pharmacology and physiology of these binding sites.

Supported by a grant from the Graduate School Research Fund, University of Washington and NIH 88 0778.

**MOTONEURON: AN ULTRASTRUCTURAL STUDY.**

M.T. Lindau and R.E. Morrisey.

Morphological evidence for regeneration of central nervous connections has been reported for the pulmonate snail Helix pomatia. In the snail, damage to the nerve supply is interrupted and, we now find, can be mediated by a variety of experimental procedures that prevent the genesis of maximal contractile tension in the closer muscle of the pincer claw. Transformation of the pincer opener muscle, an operation which restricts normal length increases of the closer muscle, also resulted in changes in the functional properties of its motor neurons. The loss of muscle tension in the pincer claw, however, is apparently not implicated in the triggering of transformation.

Supported by USPHS Research Grant NS 15006 and by an institutional grant from the Alfred P. Sloan Foundation.
FREQUENCY DEPENDENT RESPONSE PROPERTIES OF AN IDENTIFIED AUDITORY INTERNEURON IN THE CRICKET TELEOGRILLUS OCEANICUS.

Andrew Moiseff and Ronald R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14833.

The response properties of an identified auditory interneuron (Interneuron-1) were measured over a broad range of stimulus carrier frequencies (3kHz to 100kHz) for male and female T. oceanicus. Interneuron-1 was tonically excited by tones at carrier frequencies of 10kHz. The mid-threshold intensity occurred at 25kHz (61.0 dB SPL; n=10, standard deviation=5.7).

Between stimulus presentations, interneuron-1 is spontaneously active. At frequencies of 3-6kHz this spontaneous activity is suppressed (or inhibited). Suppression was most accurately measured through two-tone suppression experiments. A standard excitatory stimulus was delivered simultaneously with a test tone. The intensity of the test tone (3-6kHz) was varied until the number of spikes in response to this two-tone stimulus was one-half of the number recorded in response to the standard excitatory tone alone. Using this criterion of 50% suppression, it was determined that the peak sensitivity of the "inhibitory response" is at 52kHz (50% criterion met at 62.5 dB SPL; n=11, standard deviation=5.2).

A single ear was demonstrated to mediate the excitatory and inhibitory responses in the ipsilateral interneuron-1 (n=6), and inhibitory responses alone in the contralateral interneuron-1 (n=3).

We believe that the function of this interneuron is the detection and lateralization of ultrasonic sound signals produced by predators (e.g. insectivorous bats).


ADAPTATION OF THE TOBACCO HORNNORM (MANDUCA SEXTA) CENTRAL NERVOUS SYSTEM TO NICOTINE. Catherine F. Morris (SPON: D.L. Gilbert). NIH, Bethesda, MD 20205.

Manduca sexta caterpillars, when feeding on tobacco, chronically tolerate haemolymph levels of 2.3 X 10^-9M nicotine (Self et al., 1964, J. Ins Phys 10:907), a concentration several orders of magnitude greater than that required to alter synaptic function in some insects. This implies either that the Manduca CNS is intrinsically insensitive to nicotine or that a blood-brain barrier system operates against the toxin. In vitro electrophysiological studies (comparing Manduca to Periplaneta americana) established that Manduca CNS is susceptible to nicotine, but 2 orders of magnitude less sensitive (indicated by drug-evoked discharge monitored in connectives and ganglionic polarization measurements) than Periplaneta. During 5 min exposures, desheathed Manduca nerve cords have a threshold for response at 5 X 10^-9M, whereas that for intact cords is between 1 and 2 X 10^-8M nicotine. The cholinergic agonists muscarine, lobelline and ACh are all effective at 10^-8M or less. Studies with radioactive nicotine show: a) the blood-brain interface is not efficient in excluding nicotine passively from the CNS, since uptake ratios are the same in Manduca and the cockroach for incubations of 2-30 min b) nicotine is rapidly metabolised by Manduca's CNS to water-soluble compounds that are almost certainly conjugates of the hydrolysis product(s) of nicotine c) nicotine uptake has a saturable component which may reflect an alkaid transporting system capable of removing nicotine from extracellular spaces and d) nicotine and its CNS metabolites wash out of the Manduca CNS according to 2 exponentials, whereas 3 exponentials are found in Periplaneta, suggesting that a "nicotine space" (viz. a deep extracellular compartment) is missing in Manduca. Conjugate formation from nicotine would require a mixed-function oxidase system plus conjugating enzymes, this is unlikely. There is a limited amount of metabolic activity in the cockroach CNS indicating that the rate of detoxification relative to influx is the critical factor in conferring protection on Manduca. (Study carried out in Dept. Zool., Univ. of Cambridge, U.K.)

INTRACELLULAR pH REGULATION IN CRAYFISH NEURONS STUDIED WITH IOH-SENSITIVE MICROELECTRODES.

William Moody. University of Bristol, Bristol, BS8 1TD, England.

The intracellular pH (pHi) of crayfish motoneurons was measured using an assay for neural function. Perfusions of ethanol at concentrations of up to 2.0% caused a 2-4 fold increase in number of giant fiber responses to cercal stimulation with 30 seconds of perfusing the body cavity. The site of action was localized by means of vaseline barriers which isolated specified regions of the CNS. Only perfusion of the terminal ganglion caused an increased spike number comparable to that of wholly perfused nervous systems. Extracellular records from theafferent neurons in the cereal sensory nerve during stimulation were unaffected by alcohol treatment. Similarly perfusion of anterior connectives and segmental ganglia did not cause a significant increase of giant fiber responses to cereal stimulation. The principal site of action of ethanol appears to be in the neuropile of the terminal ganglion, and probably at the sites of cereal nerve input to the giant fiber arborizations. Spatial localization of point sound stimuli as manifested in giant fiber spike number has been quantitated and can be used as an assay for neural function. Perfusions of ethanol at concentrations of up to 2% induced increased spike number but does not affect directional response. At higher perfusion concentrations directional responses are abolished and desynchronized. The inhibitory effect of this system is not fully elucidated but the results point to an alteration in relative excitation/inhibition of the giant interneurons. This work was supported by a grant from the Alcohol and Drug Abuse Institute, Univ. of Wash.


Giant fiber activity elicited by mechanoreceptor input to identified interneurons of the abdominal nerve cord in the house cricket Acheta domestica was used as an assay for the action of ethanol on electrophysiological function of an insect sensory system. Giant fiber activity is affected by perfusion with ethanol. Concentrations below 0.20% caused a 2-4 fold increase in number of giant fiber spikes in response to cercal stimulation with 30 seconds of perfusing the body cavity. The site of action was localized by means of vaseline barriers which isolated specified regions of the CNS. Only perfusion of the terminal ganglion caused an increased spike number comparable to that of wholly perfused nervous systems. Extracellular records from the afferent neurons in the cereal sensory nerve during stimulation were unaffected by alcohol treatment. Similarly perfusion of anterior connectives and segmental ganglia did not cause a significant increase of giant fiber responses to cereal stimulation. The principal site of action of ethanol appears to be in the neuropile of the terminal ganglion, and probably at the sites of cereal nerve input to the giant fiber arborizations.
840 IDENTIFIED NEURONS OF THE STOMATOGASTRIC GANGLION HAVE DIFFERENT, CHARACTERISTIC MEMBRANE TIME CONSTANTS. Brian Mulloney, Kate Skinner, and Donald R. Edwards, Jr.,* Zoology, University of California at Davis, Davis, CA 95616.

The gastric system of the stomatogastric ganglion includes ten motor neurons and two interneurons which interact to generate a characteristic motor pattern. These neurons are identifiable in terms of the muscles they innervate, the nerves their axons travel, and the muscles they make within the ganglion (Mulloney and Selverston, 1974). As part of an effort to simulate the pattern-generating mechanism, we have measured the time-constants (τm) of the membranes of some of these neurons using Jack and Redman's (1971) brief current-pulse method, and discovered that these neurons differ characteristically in this parameter. The data on hand for these neurons are:

<table>
<thead>
<tr>
<th>Neuron</th>
<th>τm (msec)</th>
<th>Rin (Mohm)</th>
<th>Rm (Mohm cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGN</td>
<td>140</td>
<td>21</td>
<td>0.140</td>
</tr>
<tr>
<td>AMH</td>
<td>20</td>
<td>2</td>
<td>0.020</td>
</tr>
<tr>
<td>LGN</td>
<td>---</td>
<td>7</td>
<td>0.037</td>
</tr>
<tr>
<td>KNS</td>
<td>---</td>
<td>10</td>
<td>0.035</td>
</tr>
<tr>
<td>EPNS</td>
<td>14</td>
<td>15</td>
<td>0.120</td>
</tr>
<tr>
<td>GM</td>
<td>35</td>
<td>7</td>
<td>0.035</td>
</tr>
<tr>
<td>Interneuron 1</td>
<td>14</td>
<td>---</td>
<td>0.014</td>
</tr>
</tbody>
</table>

τm lets us calculate the specific membrane resistivity (Rm) of these neurons, since τm = Rm Cm and Cm = 1 μF/cm². These neurons differ up to ten-fold in their specific membrane resistivities, and therefore in the density of ionic channels.

The input resistances of these neurons were also measured, in most cases in the same microelectrodes. The values of Rin are given in the Table; Rin is not a simple function of Rm.

Chemical synaptic transmission onto these neurons should change both τm and Rin, since it will cause local changes in the membrane's conductance which our methods will integrate into closely approximate the true values. We think the highest measured values of τm most receive continuous synaptic input (Graubard, 1978; Mulloney, unpublished). We conclude that the position of a hair along the proximo-distal axis has a correlation with the membrane resistivity which is the most closest approximate the true values.

Supported by US PHS grant NS 12295.


An array of sensilla known as clavate hairs are located near the legs of the cricket. These clavate sensilla are thought to be receptor-activated Na+ and Cl- responses (Carpenter et al, J. Neurobiol. 8:119, 1977). Aschler (J. Physiol. 254:193,1972), but DTC appears to block the ionic channels common to a class of receptor-activated Na+ and Cl- responses (Carpenter et al., J. Neurobiol.). We conclude that DTC, ACh and FMRFamide are all acting at different receptors in the radula protrator muscle. (Supported by NIH grant HL-09283 to M. J. G.)
844 INSTRUMENTAL CONDITIONING IN CRAYFISH: LEVER PULLING FOR FOOD. Gen C. Olson and Robert Strandberg. Dept. of Psych., UCLA, Los Angeles, CA 90024.

Crayfish are relatively adept at manipulating objects with their claws. It would be interesting to determine if they can learn manipulative tasks. Previous studies in our laboratory have concentrated on locomotion tasks, e.g., mazes or shuttle box paradigms. This study demonstrates that crayfish can be trained to pull a lever.

845 IN TANDEM PHYSIOLOGICAL AND CHEMICAL STUDIES: USE IN IDENTIFICATION OF NEW HISTAMINERGIC NEURONS IN APLYSIA. Joyce K. Ono and Richard E. McCaman. City of Hope Nat. Med. Ctr., Duarte, CA 91010

We have modified a freeze-substitution technique used for isolating individual neurons for chemical assay to permit chemical measurements of single identified neurons. Previous studies in this laboratory have identified cholinergic, serotoninergic, and histaminergic neurons from Aplysia californica. We have assayed for their respective transmitters using electron microscopic autoradiography and freeze-substitution (Ono & McCaman, Brain Res. 165:156). The histaminergic neurons (RC2 and LC2) proved to be particularly sensitive and adversely affected by the fixation procedure used for their isolation. This freeze-substitution procedure utilized ethylene glycol (Giller & Schwartz, J. Neurophysiol. 34:93) and greatly facilitated isolation of "clean" neuronal somata. In the present study, substitution of propylene glycol for ethylene glycol in the freeze-substitution procedure permits excellent recovery of histamine (HA) from C-2 neurons subjected to intracellular recording and staining.

Additional HA-containing neurons have been discovered in the CNS of Aplysia using this new freeze-substitution procedure. These neurons are generally difficult to distinguish as individuals on the basis of visual inspection. Application of in tandem physiological and chemical techniques has resulted in the unequivocal identification of additional histaminergic neurons. The new histaminergic neurons, designated RC3 and LC3: (a) are located bilaterally on the ventral surface of the cerebral ganglion; (b) contain a concentration of HA comparable to the previously identified C-2 neurons; (c) make monosynaptic connections with some of the same follower cells of the C-2 neurons; and (d) evoke the same type of postsynaptic responses as the previously identified C-2 neuron types in these followers.

Use of the propylene glycol medium also markedly improved the recovery of dopamine (DA) and N-acetyl-3,4-dihydroxyphenyl- lionic roots from the leech, Macrobella decorata. Previous studies with the ethylene glycol medium indicated a significant loss of DA from the rootlets during freeze substitution. It is anticipated that the use of the propylene glycol freeze-substitution technique will make it possible to isolate and assay the single neurons in this species.

In tandem physiological and chemical studies of individual neurons are essential for characterizing small, visually nonde­


The lobster has dimorphic claws. The cutter claw is adapted for speed; the crusher claw is power. When these claws are lost due to injury, the animal has the ability to regenerate them. The claws regress in the form of soft buds enclosed in a chitinous capsule. At the next molt, the capsule is shed with the carapace, and a small but functional claw appears. The system has potential for experimentation relevant to neurotrophic influence on muscle development.

Opener and closer muscles of the lobster differentiate early in the development of the bud and then become acellular. Electron microscopy shows that innervation occurs early in the growth of the buds. Motor terminals appear active; they have dense bodies and well defined axons. The axons are already well equipped with mitochondria and large numbers of vesicles, dense cored as well as clear.

Muscle fibers are at first contiguous. As the bud grows they come to lie further apart with spaces between them. Individual fibers increase in diameter, and there is apparent infolding of the surface leading to fiber splitting. Cross sections of paired buds show that the opener and closer muscles of the cutter are smaller than the corresponding muscles of the crusher. The tendons are similarly unequal. In addition, the closer muscle in each bud has histoch­


The ventral nerve cord of the cockroach contains seven bilateral pairs of giant fiber interneurons (GFs), which are arranged into dorsal and ventral groups consisting of three and four pairs respectively. Analysis of branching of GFs anterior to and including the third thoracic ganglion indicates, by electron microscopy, that these neurons are essential for characterizing small, visually nonde­

mos-histochemical and ultrastructural character­

istics that identify the type of claw from which it originates. Crustacean muscle can be typed on the basis of histochemical differences in reaction to oxidative capacity by NADH-diaph­

hrase, and "fastness" by myofibrillar ATPase at pH 9.4 (Ogonowski and Lang 1978). The closer of the cutter bud, but not the closer of the crusher bud, contains fibers with high activity for ATPase at pH 9.4. However, very early cutter buds will not stain darkly for ATPase at pH 9.4, which may qualify the closer as slow muscles at that stage. On emergence, the closer fibers in the crusher bud are slow fibers of high oxidative capacity.

The sarcomeres of the "fast" fibers of the cutter bud are shorter and differ from those in slow areas by having thinner, less dense Z-lines, clear M-bands and lower filament ratios of actin : myosin.
Invertebrate Neurobiology

849 PHYSIOLOGICAL CORRELATES OF PHONOTAXIS IN FLYING CRICKETS. Gerald S. Pollack and Ronald R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Tethered, flying crickets (Teleogryllus oceanicus) perform steering movements in response to electronically synthesized models of calling songs played from loudspeakers located to the right or left. They attempt to turn toward song models with carrier frequencies similar to those found in cricket songs, but away from models with carrier frequencies that approximate that of bat echolocation cries (Nielson, Pollack and Hoy, Proc. Natl. Acad. Sci. U.S. 75, 4052-4056, 1978). We have recorded steering movements for both eyes simultaneously by recording the electrophysiological activity of relevant nerve roots and muscles.

Steering maneuvers include lateral movements of the antennae, legs and abdomen, rotation of the head, and changes in the pronation of the forewings. The movements are rhythmic and reflect the temporal structure of the song stimulus. Discrete pairs of sound pulses in the song stimulus are followed by discrete steering movements. The temporal structure of the song is evident in the firing patterns of motor neurons and muscles as well. Electrophysiological recordings show that, in some motor neurons, the song pattern is superimposed on the regular flight rhythm.

The auditory receptors which mediate sound-activated steering are located on the front legs and project to the prothoracic ganglion. Motor neurons which accomplish many components of the behavior (e.g. wing adjustments, leg and abdomen movements) are located in the thoracic and abdominal ganglia. Section of both cervical connectives eliminates sound-activated steering, even though it does not affect any direct interneurons that may exist between the auditory and motor centers mentioned above. It is therefore possible that the neurons which provide phasic sound-related information to direct motor neurons are located in the head ganglia. Alternatively, steering motor neurons may be driven in a phasic, sound-related manner by neurons from the prothoracic ganglion and the effectiveness of this pathway might be modulated by information descending from the head ganglia.

850 RESETTING THE CIRCADIAN CAP RHYTHM IN THE APLYSIA EYE BY LL TO DD TRANSITIONS, II: EVIDENCE FOR TWO TIMING MECHANISMS. Robert G. Prichard* and Marvin E. Lickey. Univ. of Ore., Eugene, OR 97403.

In another abstract we reported that activity in the optic nerve can participate in causing a phase shift (Δø) in the circadian rhythm of compound action potentials (CAP) in the Aplysia eye. We now report that (i) the timing of this nerve activity is determined by one phase (t) while the other is determined by a second (t'), and that (ii) the critical photoreceptors for determining the magnitude of Δø are in the eye itself. Aplysia were first exposed to LD 12:12 and then, beginning at dawn, switched to LL for 16, 24 or 24 h. The eyes and brain were then removed and the recording chamber, the optic nerve of one (detached) eye was cut and the entire preparation put into DD (LL/DD). After 1 to 14 h in DD the optic nerve of the other (attached) eye was also cut. On the following 2 or 3 cycles the CAP rhythm of the attached but not the detached eye showed a Δø of the predicted size provided that the nerve remained intact for a sufficient time. If the nerve was cut too soon the Δø did not occur at all; there were no partial Δø's. Thus activity in the optic nerve has an all or none effect on its attachment. The minimum required duration of attachment (Dm) following LL/DD decreased linearly as the duration of LL increased. The duration of LL plus Dm always equaled 28±1 h, i.e., the critical nerve activity always occurred at a fixed internal following the last dawn seen by the intact Aplysia. The nerve activity, therefore, must be timed by a mechanism that is initiated or reset by dawn, and that is preserved in an LL/DD. As described in the previous paper, the magnitude of Δø caused by the nerve activity establishes a 12 h phase lag between LL/DD and the mid-point of the circadian rhythm. This led us to hypothesize that perhaps the CAP rhythm itself, and the other reset by LL/DD. In a second experiment we exposed Aplysia to several cycles of LD 12:12 at dawn and then switched to LL for 16 h. The eyes of one animal (one eye detached) were then removed and to a special recording chamber in which the eyes and brain could be separately illuminated. LL was continued for 16 h in vitro with the eye and brain not both cultured. During the in vitro LL optic nerve activity was blocked with isotonic sucrose to prevent transfer of photoreceptor information between the eye and brain. The magnitude of Δø in the attached eye was determined by the LL/DD applied to the eye and not that applied to the brain. Thus, dominant photoreceptors for the second timing mechanism, and probably the mechanism itself, are in the eye. The question of how the optic efferents contribute remains unanswered. Perhaps they facilitate coupling between two oscillating neurons. NSF 29251, NS 12374.

851 ROLE OF THE PNEUMOSTOME MOTOR SYSTEM IN WATER BALANCE AND RESPIRATORY FUNCTION IN THE TERRITORIAL SLUG, LIMAX MAXIMUS. David J. Prior, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

A major problem faced by air breathing animals is the threat of dehydration due to evaporative water loss across respiratory surfaces. Many behavioral responses to dehydration result in reduction of the area of exposed respiratory surface. This however leads to a physiological dilemma: responses which reduce water loss likewise reduce oxygen uptake. Therefore such behaviors must be balanced responses to both dehydration and oxygen demand. Using the terrestrial slug, Limax maximus, we have begun a study of the neural control of the behavioral responses to dehydration and oxygen demand. In a hydrated Limax, the pneumostome is continually open. Following a specific level of dehydration (10% loss of body weight) the pneumostome begins to open and close rhythmically, an activity that can reduce both water loss and oxygen uptake. In that the onset of this rhythm corresponds with a specific level of hydration the possibility exists that the sensory cue initiating the activity is increased hemolymph osmolality. To test this hypothesis the osmolality of hemolymph from slugs at varying levels of hydration was measured. Dehydration results in an increase in hemolymph osmolality that is essentially linear. Hence this variable could be involved in the onset of the pneumostome rhythm. This hypothesis is being tested using isolated head pneumostome preparations that allow intracellular recordings to be made from ganglion neurons whose activity is correlated with opening and closing of the pneumostome. Their activity patterns are affected by varying the osmolality of the perfusion medium. An attempt is being made to see the responses of these and other neurons to saline osmolalities in the range that corresponds to the initiation of the pneumostome rhythm. Supported by NIH grants NS05856 (CHP) and NS11311 (DJP).
MORPHOLOGICAL PARAMETERS OF THE SQUID GIANT SYNAPSE: RELATIONSHIP WITH NEURONAL PATHWAYS INVOLVED IN TRANSFER OF INFORMATION RELATED TO MUCUS RELEASE. Stephen R. Kandel, Princeton University, New Jersey 08544.

FORMAL STUDIES ON THE GIANT SYNAPSE of the squid Loligo pealei (Pumphrey and Reese, Neurosci. 3,685) showed this synapse to consist of a number of active zones (AZ) defined by: 1) parallel appositions between the pre- and postsynaptic giant axons, 2) electron-dense fuzunant subjacent to both membranes and within the synaptic cleft, and 3) clusters of vesicles within the presynaptic cytoplasm. Freeze-fracture revealed patches of large, relatively-homogeneous, intramembraneous particles in the cytoplasmic leaflet of the presynaptic membrane and in the external leaflet of the postsynaptic membrane. These patches were in close contact with each other and with AZ seen in thin sections. From the freeze-fracture data, the concentration of particles at the presynaptic AZ was 1.55 x 10^17 particles/μ^2. In addition, ultra-thin sections were taken at 50μ intervals through an entire giant synapse. The length of AZ appearing in a section at each interval was plotted vs. the distance along the synapse (total distance=900μ). The curve was relatively smooth, tapering off slowly toward the distal end of the presynaptic axon. The total area of AZ (area under the curve) was 1.3 x 10^4μ^2. By plotting the distribution of lengths of individual AZ in thin sections, and assuming that these lengths were sections through circular active zones whose diameters had a normal distribution, we estimated the average AZ area as 1.25±.4μ^2. From these figures, the entire synapse contained 2.0 x 10^14 presynaptic particles in 1.1 x 10^3 active zones with 1.8 x 10^14 particles each. Assuming that a presynaptic AZ has, or contains, a voltage-sensitive channel for Ca^2+, the conductance of such channels may be calculated from the total synaptic conductancy. The presynaptic AZ contains 8.5 x 10^10 Ca^2+ conductances of 0.2 picosiemens/particle. These estimates agree with values for the Ca^2+ conductance of individual channels determined from noise analysis studies in molluscan neurons (Brown, Aakle, and Lee, Ann. N.Y. Acad. Sci.,1978). In addition, the number of postsynaptic particles is about equal to the number of presynaptic particles. From the total postsynaptic current, the calculated conductance is 1.10 picosiemens/particle, again in agreement with results of noise analysis on Na channels. The agreement between these data suggests that equating presynaptic active-zone particles with Ca^2+ channels, and postsynaptic particles with channels carrying postsynaptic current, is quantitatively reasonable.

NEURONAL PATHWAYS INVOLVED IN INFORMATION RELATED TO ACQUISITION LEARNING IN THE COCCinelLA americana. Roger R. S. Pumphrey and S.M. Eisenstein, Zoology and Biophysics Departments, University of Pittsburgh, Pittsburgh, PA 15260.

Behavioral experiments have shown that transfer of information related to leg position learning occurs via the interganglionic connectives. Anatomical and physiological experiments were done to identify specific pathways by which information transfers from the prothoracic to the mesothoracic ganglion. These experiments were done using free choice and light stimulation. The results showed that there are 2.0 picosiemens/particle, again in agreement with results of noise analysis on Na channels. The agreement between these data suggests that equating presynaptic active-zone particles with Ca^2+ channels, and postsynaptic particles with channels carrying postsynaptic current, is quantitively reasonable.


A central neuronal pattern generator underlies movements of buccal musculature used in feeding by the terrestrial mollusk Limax maximus. The activity of the pattern generator is independent of sensory feedback. However, feeding activity in intact animals and feeding motor programs (FMP) in isolated nervous systems can be modulated by changes in food-sensed related stimuli. We examined the effects of load on buccal muscles, concentration of food attractant, and feeding activity in intact animals and FMPs in isolated preparations. To monitor feeding movements in intact animals, pellets of prepared food in 3% agar base were attached to the animal by a multilink transducer. Sequences of individual "bites" were recorded and data analyzed in terms of instantaneous bite frequency throughout a meal. Meal duration varied from 3 minutes on 8% agar and amount of food extract in a pellet, hardness and chemostimulus quality of food were varied, and the effects on feeding movements were examined. In isolated preparations consisting of buccal and cerebral ganglia with attached chemoreceptive lips, delivery of food extract to lips triggered bursts of FMF, while stimulation of mucus release was reduced by extracellular electrodes. Innervated buccal muscles or esophagus and crop were included in some preparations. Effects of FMF on load on buccal muscles, concentration of food extract, or degree of esophagus/crop inflow were examined.

LOAD. Increasing hardness of a food pellet increased load on feeding apparatus in an all-or-none manner. When pellets were attached to the animal, a simple threshold, spontaneously active motor neurons which are not coupled electrically and inking controlled by high threshold motor cells that are coupled electrically within the presynaptic AZ with Ca^2+ conductance of 0.2 picoemission/particle. These estimates agree with values for the Ca^2+ conductance of individual channels determined from noise analysis studies in molluscan neurons. The activity of the pattern generator is independent of sensory feedback. However, feeding activity in intact animals and feeding motor programs (FMP) in isolated nervous system preparations can be modulated by changes in food-sensed related stimuli. We examined the effects of load on buccal muscles, concentration of food attractant, and feeding activity in intact animals and FMPs in isolated preparations. To monitor feeding movements in intact animals, pellets of prepared food in 3% agar base were attached to the animal by a multilink transducer. Sequences of individual "bites" were recorded and data analyzed in terms of instantaneous bite frequency throughout a meal. Meal duration varied from 3 minutes on 8% agar and amount of food extract in a pellet, hardness and chemostimulus quality of food were varied, and the effects on feeding movements were examined. In isolated preparations consisting of buccal and cerebral ganglia with attached chemoreceptive lips, delivery of food extract to lips triggered bursts of FMF, while stimulation of mucus release was reduced by extracellular electrodes. Innervated buccal muscles or esophagus and crop were included in some preparations. Effects of FMF on load on buccal muscles, concentration of food extract, or degree of esophagus/crop inflow were examined.

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Recent evidence indicates that the 14 giant interneurons (GIs) of the cockroach Periplaneta americana can excite motor neurons with direct movement of the metathoracic (T3) legs (Ritzmann and Camhi, J. comp. Physiol. 125:305-316, 1978). Each of the GIs is excited by a specific set of wind directions (Westin et al., J. comp. Physiol. 139:11-15, 1978). The spatially specific wind input and the motor output of each GI suggests that they may play an important role in directing the wind mediated escape behavior (Ritzmann and Tom. J. comp. Physiol. 125:305-316, 1978). Moreover, when a wind stimulus from any direction will excite 8-12 GIs simultaneously. Therefore, in order to understand how the GIs function in response to natural stimuli, we must determine the output of the cells combine when more than one GI is excited.

With this in mind, I have investigated the motor response to intracellular stimulation of pairs of GIs. The GIs were impaled with microelectrodes filled with 4% Procion yellow as in previous experiments (Ritzmann and Camhi, 1978). They could then be stimulated with high frequency trains of current pulses while motor activity was monitored with extracellular electrodes on nerve branches 5rl (containing primarily depressor axons) and 6Br4 (containing primarily levator axons) in one of the metathoracic legs. Subsequent to the recording session Procion yellow was infused into the GIs for histological identification. A paired T-test was used to compare the motor response to stimulation of each GI individually and the response to stimulation of both GIs together.

When two dorsal GIs were stimulated, the motor activity was augmented. For example, GI-5 excites primarily depressor motor neurons, while GI-7 excites both levators and depressors. When GIs 5 and 7 were both stimulated the resulting depressor activity was greater than that from either GI alone. Moreover, when the GIs were paired, less activity in either GI was required to elicit a motor response when than when they were stimulated individually. A similar situation occurred when two ventral GIs were paired. GI-22 and the output of the dorsal GI. This suggests that then direct movements of the T3 legs (Ritzmann and Camhi, 1978). They could then be stimulated with high frequency trains of current pulses while motor activity was monitored with extracellular electrodes on nerve branches 5rl (containing primarily depressor axons) and 6Br4 (containing primarily levator axons) in one of the metathoracic legs. Subsequent to the recording session Procion yellow was infused into the GIs for histological identification. A paired T-test was used to compare the motor response to stimulation of each GI individually and the response to stimulation of both GIs together. Rather, they may represent two distinctly separate pathways. Supported by NSF Grant BNS-78-06192.

EFFECTIVE NEURONS TO THE RETINA OF OCTOPUS: ANATOMY AND PHYSIOLOGY. William M. Salzberg (SPON: T.M. Bullock). Dept. of Neurosci., Sch. of Med., UCSD, La Jolla, CA 92037.

The eye of Octopus bimaculoides is connected to the ipsilateral optic lobe by about 150 small retinal nerves. Each nerve contains both centripetal and centrifugal fibers. Using the proximal stump of a cut nerve as a wick for cobalt staining, the relationship within the optic lobe between photoreceptor axon terminations and the cell bodies of neurites whose frequency depends on the amplitude of the step. Reverse steps cause a frequency decrease whose size is proportional to the amplitude of the step and also prior activity. Activity from these cells can be distinguished from that of photoreceptors by their shorter (1.5 vs. 5 ms) spike duration. Efferent cells show responses to visual, tactile or otocystal stimulation. Steps of increasing light cause a delayed, transient spike frequency increase followed by a steady state waveform whose frequency depends on the amplitude of the step. Reverse steps cause a frequency decrease whose size is proportional to the amplitude of the step and also prior activity. Hysteresis occurs, depending upon prior retinal adaptation.

A water stream or stroking the mantle or wab produce two kinds of responses: a transient burst or suppression of background activity. Suppression also spontaneously followed a mantle movement resembling stimuli seen in the wild. Bursts of spikes were induced by vibrating the experimental chamber and by angular rotation of the preparation presumably via chemical changes in the wild. Golgi material indicates that these cells terminate at or near photoreceptor inner segments. The anatomy and physiology of these cells suggest that a visual input receptor factor cells of the retina to a restricted portion of the optic lobe and back to the retina.

This work was supported by NSF and NIH grants to T.M. Bullock.

OPTICAL SPIKES FROM A SALIVARY GLAND. B.M. Salzberg and S.M. Seneame, Dept. of Physiology and Pharmacology, School of Dental Medicine, Univ. of Pennsylvania and Monell Chemical Sensors Center, Philadelphia, PA 19104.

The mechanism(s) underlying excitation-secretion coupling in gland cells is not presently understood. The small size of many of these cells has limited the utility of conventional intracellular microelectrode recording. Since optical measurement of membrane potential has proven possible in giant axons, invertibrates central nervous system and cardiac muscle, as well as a number of other preparations, we hoped that these techniques could be used to study electrical phenomena in gland cells. In order to facilitate optical recording, the salivary gland of the snail Helisoma trivolvis has been shown to produce overshooting action potentials and, in the experiments to be reported here, the Merocyanine-oxazolone dye NK 136 was used to record extrinsic voltage dependent absorption changes from extended regions of this gland while electrical activity in a single ascinar cell was monitored simultaneously with a microelectrode. Merocyanine-oxazolone, NK 136 is visible in a single oscilloscope sweep could be obtained whether the gland was superfused or perfused through the lumen with a Ringer's solution containing the dye. The wavelength of the optical signal differed significantly from that exhibited by this dye when applied to voltage clamped squid giant axons or barnacle supraesophageal ganglion cells, but was similar to that found with a close Merocyanine-rodamine analogue (Dye XVII) in cardiac muscle. The largest signals were recorded at 590 nm, where there was an increase in absorption by the stained gland. Decreases in absorption were recorded at 540 nm and 720 nm in squid giant axons, a depolarization resulted in a decrease in absorption at all wavelengths.

We attributed some observed variability in the amplitude of the optical signal to differential invasion of the gland, and, in order to study this effect, we made two sets of experiments. In the salivary gland was monitored optically for 50 seconds at a time while intracellular microelectrode recordings were made from the primary salivary gland effector cells. Compared with its bilateral homologue, activity in the contralateral effector neuron seemed to evoke electrical changes in the gland which were accompanied by smaller optical signals. Supported in part by NSF Grant BNS-770502, NIDR Grant DE-05271 and BRS Grant RR-05337-17.

Effects of trimethadione (TMO), the prototypical drug for treatment of petit mal epilepsy, were examined on somatic action potentials in neuron R2 of the abdominal ganglion of Aplysia californica. Intracellular stimulation and recording was accomplished by inserting two 3M KCl filled glass microprobes into RT.

In artificial seawater (ASW), TMO (10 μM) increased the action potential regularization time (RT) of neuron R2, in the presence of TTX, cell L10 is voltage-clamped from a holding potential of -60mV graded transmitter release can be evoked by graded depolarizing command pulses in the range of membrane voltage (-55mV to -35mV) in which it is less to increasing. Depolarizing the holding potential of L10 results in increased transmitter output (Shimahara & Peretz, 1978; Nicholls & Martin, 1978). The mechanistic mechanism for this form of plasticity. First, depolarization inactivates some K+ channels so that command pulses recruit a smaller K+ current. In unclamped cells, the change in the membrane potential causes spike-broadening and/or increases influx of Ca2+ during each spike (see Klein and Kandel, 1978). Second, small depolarization around resting potential (-55mV to -35mV) activates a steady-state Ca2+ current which also may contribute to the modulation of transmitter release, since even with most presynaptic K+ currents blocked (using Ba+2 substitution for Ca2+ and 4-AP), varying holding potential still affects transmitter release (see also Nicholls & Wallace, 1978). In contrast, under these conditions, the transient inward Ca2+ current evoked by depolarizing command steps is relatively unchanged from various holding potentials.

The output of cell L10 undergoes presynaptic inhibition in response to sensory stimulation. Since inhibition is blocked by concentrated K+ solutions or other substances that inhibit the Ca2+ current, the results were obtained when the set position of the tibia was altered at 15-30° intervals. (a) In some non-spiking neurons that affect tarsal motor neurons, the tonic change in membrane potential was 0-10 mV, but in others that affect tibial motor neurons, the change was only 1-2 mV. (b) Differnet inhibitory pathways were different to different ranges of tibial movement; e.g., some showed the greatest tonic changes when the PTA was re-set between 90° and 160°, others when the PTA was reset between 90° and 0°.

The other two forms of synaptic plasticity are (1) by direct steady-state activation of Ca2+ channels at more depolarized membrane potentials and (2) by transmitter-mediated decrease of Ca2+ current. Our results show that these two forms of synaptic plasticity are modulated by changes in the Ca2+ current that contribute to the modulation of transmitter release. This current can be controlled in at least three ways: (1) by membrane potential control of available Ca2+ channels that can broaden or narrow spikes and thereby increase or decrease modulation of transmitter release, (2) by direct steady-state activation of Ca2+ channels at more depolarized membrane potentials, and (3) by transmitter-mediated decrease of Ca2+ current. Supported by a grant from the Epilepsy Research Foundation.

ORDERLY SEQUENCE OF POLYSYNAPTIC SENSORY INPUTS TO CRAYFISH TAILFIP MOTOLOGUES. Dynae M. Sherwood and Jeffrey E. Wine. Dept. Psych., Stanford U., Stanford, CA. 94305

The only tailflip command neurons that are excited by sensory stimulation in the isolated crayfish abdomen are the lateral giants (LGs) (Wine & Krause, 1972, J. exp. Biol.56:1). Following an LG command, all of the efferents to the fast flexor muscle mass can be excited by the LG command. However, sensory inputs to the motor neurons of the hind legs (1,2,3). This study examines the relationship between changes in the membrane potential during the movement of the terminal potenti,
THE ANATOMICAL ORGANIZATION OF CRAWFISH SEGMENTAL GANGLIA.

The structural organization of crustacean ventral cord has been investigated using electron histofluorescent and sectional silver-stained by the method of Rowell, of Procambarus clarkii thoracic and abdominal ganglia. The basic pattern of longitudi-nal tracts, cross-ganglion commissures and vertical tracts is being mapped. The major groups of cell bodies and the central projections of the peripheral nerves will be localized in terms of these landmarks pathways. The segmental ganglia are built on a sandwich plan of alternating longitudinal and commisural bundles of axons which form tracts through the most dorsal longitudinal tract contains the giant fibers and has the largest cross-sectional area. The most ventral longitudinal tract layer is the smallest in cross-sectional area. There are alternate commissures which cross the midline of the ganglion in four broad bands or layers. Between commissural layers three and four, in the ventral part of the ganglion, lies a dumbbell-shaped mass of very finely textured neuropil which may be homologous with the "ventral association center" of insect segmental ganglia (Pipa et al., 1959; Gregory, 1974; Tyrer, pers. com.). It is not yet known whether the layers carry comparable numbers of fibers or whether the neuropil masses can be assigned behavioral or physiological functions.

The histological maps will make it possible to localize identified neurons more precisely within the ganglion (Stretton and Kравит, 1973), to test the constancy and variability of individual neurons relative to stable "addresses" within the neuropil, to test ideas about the functional layering of inverte-brate ganglia, and to propose homologies between related taxa. This work was supported by NSF grant BNS 78-10516 and US PHS grant NS 12295.


Motoneurons to feeding musculature in Navanax have been exten-sively characterized; however, the firing pattern of these motoneurons during feeding has not been studied. To understand the neural generation of feeding behavior, it is first necessary to describe the behavior. To this end, we have filmed feeding in Navanax. We have addressed ourselves to three questions:
1) What is the sequence of events in feeding? 2) Is the sequence a fixed action pattern, or can components of feeding occur without the entire sequence? 3) Is the sequence sufficiently unaffected by dissection that a feeding preparation is suitable for electrophysiological study?

Feeding begins with slow protraction of the pharynx, followed by expansion of the anterior pharynx and flaring of the lips around the prey. Removing prey during protraction or flaring stops feeding. Prey engulfment begins with a fast, lunging protraction, followed by lip closure that seals prey in the anterior pharynx. Engulfment continues with pharyngeal expansion and retraction. Removal of prey during sealing alters this sequence; retraction occurs without expansion. Expansion produces negative pressure, pulling prey into the pharynx. Holding the prey in place at the anterior pharynx does not eliminate posterior expan-sion; however, a second expansion superimposed upon the first may occur. Expansion is followed by contraction of circumferen-tial muscles. There are both inward- and outward-directed waves of circumferential contraction around prey, as well as more localized movements. Motoneurons producing movements reminiscent of those observed in specific phases of feeding have been identified.

Essentially identical sequences of movement have been observed in animals in which progressively larger segments of body wall were removed for better visualization of the pharynx and CNS. Successful prey engulfment occurs even in radically dissected preparations. This insensitivity to operative procedures allows for recording from muscles and nerves in normally feeding prepar-a tions, which should confirm the functional role of specific motoneurons.

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DGS is a McKnight Scholar in Neuroscience.

LOCALIZATION OF Dopamine in the Gill of Aplysia: some Physiological Implications. John W. Swann, Martha G. Pierson*, and Arnica Dahlstrom*. Neurobiology Department, Armed Forces Radiological Research Institute, Bethesda, MD 20014 and Institute of Neurobiology, University of Gothenburg, Gothenburg, Sweden.

PHYSIOLOGICAL IMPLICATIONS. John W. Swann, Martha G. Pierson*, and Arnica Dahlstrom*. Neurobiology Department, Armed Forces Radiological Research Institute, Bethesda, MD 20014 and Institute of Neurobiology, University of Gothenburg, Gothenburg, Sweden.

The gill of Aplysia contains 3 µg of dopamine (DA) per gram of tissue. Physiological experiments utilizing the semi-intact gill preparation led one of us (JWS) to hypothesize that DA may be a neurotransmitter for both motor and modulatory neurons thought to innervate the gill musculature (Swann et al., Br. Res. 157: 167, 1978). Additional experiments have suggested that Lg motor neurons are dopaminergic and that Lg motor neurons (Swann et al., Neurosci. Letters 10: 275, 1978). In experiments reported here, the distribution of DA within the gill is examined anatomically using fluorescent and histochemical for catechol- amines. The Hillarp Falck technique was used.

Green fluorescing nerve fibers, with the same emission spectrum as the DFA, are distributed throughout the gill. The notable exception of the bundles of longitudinal muscle fibers of the efferent vessel. Green varicosities, indicative of DA nerve terminals, cover muscle fibers in four areas of the gill: the pinnules, the efferent vessel trunklets and the circular muscles of the efferent vessel. These areas cells on the efferent vessel when small quantities of dopamine are added to a gill perfusate. Furthermore, the exception of the circular muscles of the efferent vessel, all of them contract upon activation of Lg cells. These observations support the contention that DA is a neuromuscular neurotransmitter in the gill and that Lg cells are dopaminergic.

Contractions of the longitudinal muscle fibers of the efferent vessel are initiated by firing motor neuron L9G. These effects are greatly enhanced by perfusing with gill with DA. Since these muscle fibers are not innervated by DA-containing nerve fibers, dopaminergic modulation of L9G contractions cannot be mediated physiologically by dopa-minergic modulatory neurons as originally proposed. Instead, DA modulation of L9G contractions may be humoral in origin. We have found that a major portion of the DA content of the gill is located in highly fluorescent structures which are in close association with the micro- vascularization of the gill. The cellular origin of this potentially humoral source of DA will be discussed.
870 LOCUST DORSAL OCCELLS DETECT HORIZON DURING FLIGHT. Charles P.

868 IDENTIFICATION AND MORPHOLOGICAL VARIABILITY OF A PEPTIDERIC

We are investigating the control over the release of a specific peptide hormone by studying the individual neurons responsible for its production in the tobacco hawkmoth, Manduca sexta. The hormone - bursicon - functions to trigger the sclerotization of the wing vein cuticle following adult emergence. In response to combined hormonal and neural inputs, the peptide is released from specific segmental abdominal nerves in D. melanogaster. The release is rapid -10 min. - and predictable - within 2 min. of a behavioral marker (Reynolds et al, 1979). This unit may represent a common neural pathway for roll orientation information from the head to the neck and thorax. Abdominal connective fibers (ACFs) were divided into two groups depending upon their effects on the intracellular activity of the f5 and f6 neurons. First category ACFs elicited DPs in those neurons whose dendritic trees were located ipsilateral to the stimulated ACF. These DPs followed the stimulus with a fixed delay up to 50 Hz. The amplitude of the DPs varied with the stimulus frequency. The second category of ACFs included those whose stimulation evoked DPs or IPSPs in f5 and f6 neurons which were not phase-locked with the stimulus. These fibers, which correspond to the extension and flexion command fibers (CFs) described in crayfish by Evoy and Kennedy (1967), produce strong bilateral effects when stimulated. Some fibers (extension CFs) elicit IPSPs in f5 neurons and an increase in DPs in f6 neurons while others (flexion CFs) produce an increase in DPs in f5 neurons and an apparent inhibition of DPs in f6 neurons. Supported by NIH Grant 12262 and a Predoctoral Fellowship.


The five excitatory motorneurons and the peripheral inhibitory which innervates the superficial flexor muscles (SPMs) in each half-abdominal segment of the lobster, Homarus americanus, are identified using neurophysiological and cobalt staining techniques. Intracellular recordings from the cell bodies of the peripheral inhibitory (f5) and the largest motorneuron (f6) reveal three kinds of potentials: subthreshold depolarizing potentials (DPs), IPSPs and attenuated action potentials. Abdominal connective fibers (ACFs) were divided into two groups depending upon their effects on the intracellular activity of the f5 and f6 neurons. First category ACFs elicited DPs in those neurons whose dendritic trees were located ipsilateral to the stimulated ACF. These DPs followed the stimulus with a fixed delay up to 50 Hz. The amplitude of the DPs varied with the stimulus frequency. The second category of ACFs included those whose stimulation evoked DPs or IPSPs in f5 and f6 neurons which were not phase-locked with the stimulus. These fibers, which correspond to the extension and flexion command fibers (CFs) described in crayfish by Evoy and Kennedy (1967), produce strong bilateral effects when stimulated. Some fibers (extension CFs) elicit IPSPs in f5 neurons and an increase in DPs in f6 neurons while others (flexion CFs) produce an increase in DPs in f5 neurons and an apparent inhibition of DPs in f6 neurons. Supported by NIH Grant 12262 and a Predoctoral Fellowship.
872 LOCUST OVIPOSITION: A SYSTEM FOR THE STUDY OF MOTOR FUNCTIONS; THE VENTRAL PAIR LEVERS THE ABDOMEN DOWN LENGTH OF 2.7 CM. TO MIDDLE PROCESSES WORK BY A DIVERGENT MOTION INSTEAD OF WHICH ARE UNIQUE AMONG INSECTS, SINCE THE FOUR TERMINAL APPENDAGES ARE EROSION PRODUCTS (Vincent, J. F. 1975) J. Ent. 150:175-181). SPECIALIZATIONS FOUND ONLY IN THE FEMALE ALLOWS THE MOTIVE FORCES OF THE OVIPOSITOR TO STRETCH THE ABDOMEN FROM A NORMAL LENGTH OF 2.7 CM. TO 0.3 CM. (DATA APPLY TO ABBREVIATED NITRITIC.)

A SINGE GANGLION OF THE LOCUST NERVOUS SYSTEM, THE TERMINAL ABDOMINAL GANGLION, IS CAPABLE OF DRIVING THE DORSAL AND VENTRAL VALVES OF OVIPOSITOR TO STRETCH THE ABDOMEN FROM A NORMAL LENGTH OF 2.7 CM. TO 0.3 CM. (DATA APPLY TO ABBREVIATED NITRITIC.)

The movements of the ovipositor valves are entirely controlled by bilaterally symmetrical muscles. The ventral valves, sternal appendages of the eighth segment, are innervated by the 8th sternal nerves. The dorsal valves, sternal appendages of the ninth segment, are supplied by a serially homologous pair of eighth sternal nerves. The somata and dendritic fields of the neurons whose axons travel in these nerves are compartmentalized into two separate ganglionic regions. This behavior can only be elicited in sexually mature adults. Extracellular nerve and muscle recording, 1975a, were made and an autonomic cycle motor pattern which is comprised of two major classes of alternating burst activity. These experiments were designed as a framework for intracellular analysis of the mechanisms underlying initiation and maintenance of oviposition behavior. Supported by N I H S 1 T 3 2 WH 0 73 5 2 7 5 7 5.


In Aplysia, the convulsants penicillin (Pen G) and pentylenetetrametrol (P T P) attenuate chloride-dependent responses to iontophoretically applied transmitter. At the same concentrations, they do not affect sodium-dependent responses. This attenuation of chloride-dependent responses is independent of the transmitter used to elicit the response, suggesting that it is acting directly with the chloride ionophore, rather than the transmitter-receptor complex (Science 197:912, 1977).

Individual sodium and chloride channels were studied by examining the current fluctuations produced by the application of acetylcholine (ACh). Steady-state responses were elicited by iontophoretic application of ACh to neurons of the pleural and pedal ganglia of Aplysia californica. Currents were recorded utilizing a single microelectrode voltage clamp which was modified to low noise measurements. Frequency response studies indicated that the clamp was effective to at least 400 Hz.

The elementary conductance of a single channel was determined from the relation gcl = 1/Iv-Venv, where the elementary current I = variance of current fluctuations/mean current. The elementary conductance of a chloride channel is 8 x 10^-12 ohm^-1 at 21°C. Pen G and P T P reduced gcl in a dose-dependent fashion; the decrease in gcl paralleled the decrease in mean steady-state current evoked by ACh application. Concentrations of Pen G (10 µM) and P T P (5 µM) which reduced the elementary conductance of chloride channels had little effect on the sodium channel, although higher concentrations of these drugs did produce a reduction in sodium channel conductance.

This data confirms previous studies that Pen G and P T P decrease transmitter-evoked chloride conductances, at concentrations which spare sodium responses. It also supports the hypothesis that Pen G and P T P attenuate responses by interacting directly with, and possibly physically blocking the chloride channel.

873 CORRECTION OF TURNING BEHAVIOR AFTER UNILATERAL CERICAL ABLATION IN THE COCKROACH. Roga Varadi* and Jeffrey M. Camhi. (Spon. F. Delcomyn). Section of Neurobiology and Behavior, Cornell, Ithaca, NY 14853.

Cockroaches (Periplaneta americana) respond to minute wind currents by turning their bodies. We describe a turn elicited by front wind from either the right or the left by turning to the left. During this time, the cockroaches did not regenerate a new cercus or any new cercal hairs. A few animals molted and developed a cercal bun which we observed within two days. Prior to cercal ablation, all animals turned away from the source of wind stimulation. One day after ablation, on most trials, the animals responded to wind from either the right or the left by turning to the left. This presumably results from the fact that of the 7 GI's on each side receive most of their sensory activation from the ipsilateral cerci. Thus removing the cerci results in the right GI's more responsive than their left homologs. By one month the animals had corrected their turns in response to wind from the left. In most of the trials these animals made turns to the right in response to wind from the left. The response was highly significant (2 test; x^2 test).

To examine the cellular mechanisms of the behavioral correction, intracellular recordings are from identified GIs before, during and after the period of behavioral correction. Immediately before and after ablating one cercus, certain ipsilateral GI's become nearly or totally unresponsive to wind. These include GI's 1 and 3. So far, we have recorded only from these two GI's after long term behavioral correction. One GI and three GI's from each side about 1/3 the number of action potentials seen in normal unablated animals. The directionality of these cells was the same as in normal animals. This enhanced responsiveness of these GI's following ipsilateral cercal ablation could contribute to the observed correction of the turning behavior.

Supported by NIH grant NS 09063 and NSF grant BMS 79-09663.


The behavioral paradigm that we have used is based on Pavlovian fear conditioning (Rescorla, 1967). 'Paired' animals (N=18) received a conditioned stimulus (CS, shrimp extract delivered to the oral veil) for 90 sec. Sixty sec after the onset of the CS, a noxious stimulus (US) was applied to the head. The US was 30 sec of pulsed electric shock (400 ma) delivered through sea water via spanning electrodes. Explicitly 'unpaired' controls (N=12) received neither CS nor US. Training consisted of 3 trials per day for 2 days. The intertrial interval was 60 sec, and 6 trials per day after the 1st day. The CS was delivered for 60 sec and then escape locomotion was triggered with a weak (50 ma) shock applied across the tail. Escape was measured as mean number of steps taken in 5 min following tail shock. Paired animals showed significantly greater escape (x̅=11.22 steps) than unpaired (x̅=2.4, p<0.005) or untrained (x̅=4.2, p<0.005) animals. This experiment replicated and extended the same results were obtained using blind training in addition to the blind testing procedure: paired animals (N=6) showed greater escape (x̅=13.23 steps) than untrained (x̅=7.13 steps) (x̅=2.13, p<0.005). Thus, specific temporal pairing of the CS with a noxious US endows the CS with properties which can subsequently facilitate a change in the animal's behavior, even after training with CS or US alone does not produce facilitated escape.

These results indicate that Aplysia can form a powerful, temporally specific association between a chemical CS and an aversive US. That the learned association can be demonstrated by testing the effects of the CS on a response system not directly involved in the training (escape locomotion to tail stimulation) supports the idea that associative learning exhibited by Aplysia may be analogous to 'learned fear' produced in vertebrates by Pavlovian fear conditioning.

Several other forms of associative learning have now been described in gastropod molluscs, including avoidance learning, baited escape, and intradose reinforcement (Gritsos & Collins, 1975; Gelperin, 1975; Crow & Alkon, 1978). Thus, it may be possible to achieve cellular analyses of different types of associative learning in gastropods and to delineate the relationships between each other and to nonassociative learning, within both a single species and across related species.
878 CHARACTERIZATION OF COMMAND INTERNEURONS EVOKING ABDOMINAL MOTOR OUTPUT TO A COCKROACH LEG IN RESPONSE TO WIND FROM DIFFERENT DIRECTIONS. Joanne Westin* and Roy E. Ritzmann, Dept. Biol., CWRU, Cleveland, OH 44106 (SPON: Leo S. Demski) 

Giant interneurons of the cockroach Periplaneta americana are thought to be involved in triggering and orienting the cockroach's escape from a wind source. It is likely that several CFs are driving an escape motor and that the ones involved vary with wind direction. Evidence for this is: 1) For any given wind direction 6-12 CFs are excited, and the ones excited vary with wind direction. The CFs are characterized by loci-specific rostrally or caudally 

addition to extension; these loci correspond to those mapped by 

Camhi and Tom, . J. comp. Physiol. 128, 193-201, 1978). 2) For turns of different directions, different leg motor neurons must be excited in any given leg (Gambhi and Tom, J. comp. Physiol. 128, 193-201, 1978). My work is designed to determine which GIs are necessary to produce the motor outputs which turn the animal away from wind from different directions.

I recorded the responses of depressor motor neurons (in nerve branch 5r1) and levator motor neurons (in nerve branch 6b4) of a metathoracic leg to wind puffs from different directions before and after cutting the ipsi- or contralateral half of the nerve cord. In an intact animal the output from depressor motor neurons to the leg is generally greater when wind is from the contralateral rear than when it is from the contralateral left. The output of levator motor neurons is generally greater when wind is from the ipsilateral rear when wind is from the ipsilateral left. These results are as expected since a wind puff from the left rear generally produces an initial depression of the left metathoracic leg followed by a lefthand return to the right metathoracic leg (Camhi and Tom, 1978).

Sectioning the ipsilateral side of the nerve cord greatly reduced the output of the leg by wind from the ipsilateral rear, while having little effect on activity in the levators caused by wind from the contralateral rear. The converse is also true. Thus, CFs from ipsilateral GIs (and/or smaller ipsilateral interneurons) provide the major input to the depressor motor neurons of the metathoracic leg when wind is from the ipsilateral rear and the ones excited vary with wind direction. In addition, CFs from contralateral GIs (and/or smaller contralateral interneurons) provide the major input to the levator motor neurons when wind is from the contralateral rear. Experiments are now in progress involving elimination of individual identified GIs.

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879 A SELF-INHIBITORY SYNAPTIC POTENTIAL ALTERS INITIAL FIRING FREQUENCY, BUT NOT SYNCHRONY, OF ALYSIA BUCCAL GANGLIA INTERNEURONS. Roy L. White* and Daniel Gardner, Dept. of Physiology, Cornell Univ. Medical Coll., New York, N.Y. 10021

Each of the two buccal ganglia of Alysia contains a coupled pair of identified motor neurons monosynaptically connected: one cell is characteristically inhibits itself, producing a self-inhibitory synaptic potential (SISP) after every action potential (AP). The SISP decays with repetitive stimuli, but does not decay with 43 msec time constant (Gardner, J. Physiol. 264:883, 1977).

As prelude to AP frequency studies, we measured synaptic current in response to conditioning APs of varying magnitude. SISP amplitude increased with increasing conditioning AP frequency (F-I) curves in both SW and dTC show single-peak responses over the range 5-18 nA, with average threshold 7 nA. The SISP increased in magnitude over 1-3-fold without affecting firing rate at the end of each train. The two cells in each ganglion receive common input and are electronically coupled (White and Gardner, Science 173:550, 1971). Blocking the SISP with dTC reduces the hyperpolarizing component of the electrotonic coupling potential without affecting coupling coefficient (0.135). Two similar motor neurons were studied in one ganglion. To determine the effect of the SISP on the firing of cell pairs, we depolarized cells near threshold with 10 sec constant-current steps injected through 2 electrodes separated by 10-20 microamps. Cross-correlation histograms from early or late times in the epoch with or without dTC were identical: a strong peak around t=0, with a slow decay to a smooth component. SISP amplitude and the depolarizing component of the coupling potential.

We conclude: 1) The SISP prolongs initial ISI, providing an analog to accommodation and ensuring a constant firing frequency for constant input current. 2) Neither the more regular firing nor the larger hyperpolarizing component of the coupling potential produced by the SISP contributes markedly to the firing of these coupled neurons.

Supported by NHI-NINDS: post-doctoral fellowship NS05971 to RLW, and research grant NS11555 and RCDM NS00003 to DG.
CONSTANCY AND VARIABILITY IN SYNAPTIC CONNECTIONS BETWEEN IDENTIFIABLE NEURONS. Jeffrey J. Wise & Grace G. Haginawa, Department of Psychology, Stanford University, Stanford, CA. 94305

Variability of synaptic connections was recently demonstrated in invertebrates (Pearson & Goodman, J. Comp. Neurol., 184, 141, 1979). We tested the hypothesis that synaptic connections among neurons are variable to the extent that they are redundant. By a redundant connection we mean one which has a higher probability of transmission than the safety factor for transmission. Redundancy is a graded property, such that a very weak synapse in parallel with a very strong synapse is intermediate in effectiveness. Remarkably similar patterns of degeneration were seen in both cockroaches and grasshoppers. We have evidence for redundant synapses in the crayfish central nervous system and find that such synapses are often missing. In contrast, synapses judged to be crucial for behavior are invariably present in the population we tested. Two sets of giant neurons were studied. A pair of giant interneuron axons (LG axons), which form electrical synapses with each other and with each axon contacts one member of a pair of giant motoneurons (MoGs). An impulse in one LG axon invariably excites the other LG axon and thus excites both MoGs directly, and one indirectly via the contralateral LG axon. The regions of synaptic contact between the LG axons and the MoGs can be visualized by freezing the MoGs in a cast of fluid silicone rubber. In prior studies, where a perfect correlation was obtained between anatomical and electrophysiological measures of synaptic transmission (Mittenthal & Wise, Nature, 233, 173, 1973), we saw extensive synaptic contact between ipsilateral LG and MoG axons, and occasionally saw adjacent branches of the MoG to the contralateral LG axon. We have now backfilled a large number of MoGs (n = 52) and intensified the wholemounts with a modification of the Timm's technique. We found connections with the ipsilateral LG axon in 100% of these ganglia, and with the contralateral LG axon in 33% of the ganglia. Homologous sets of LG axons and MoGs exist in the thre anterior abdominal ganglia. By ganglion, the percentage of crossed redundant connections was: G1: 28% (9/33), G2: 44% (8/18), and G3: 25% (4/16). We conclude that the motoneuron homologs can make synaptic connections with both ipsilateral and contralateral LG axons. The strong, ipsilateral connection has a high probability of functioning, while the redundant, contralateral connection can usually be omitted. Since the strong coupling between the LG axons renders the contralateral connection redundant, its absence should have no behavioral consequences. We suggest that nervous systems may be overconnected to a significant extent, and that redundant connections will tend to be variable, since natural selection may either eliminate or reinforce them.

Supported by NSF Grant BNS-78-14179. JWM is an Alfred P. Sloan Research Fellow.


Although many vertebrate sensory systems are somatotopically organized, the organization of afferents in invertebrates remains largely unexplored. Several early observations suggest that afferents may be discretely organized in insect peripheral nerves. Wigglesworth noted that sensory axons, which develop from cell bodies in the epidermis, follow preexisting afferents in their path to the central nervous system. Edwards and Palka found that removal of the cercus tip in crickets resulted in a definite area of degeneration in the central nervous system. These studies imply that afferents from a defined body surface gather together and remain grouped in peripheral nerves.

On this group of afferents produce an organized organization of afferents? To answer this question we took advantage of the fact that insect axons degenerate rapidly after separation from their cell bodies and produce changes detectable by light and electron microscopy. Degenerative changes include clumping of axonal organelles, separation and expansion of glial wrappings and final resorption of axons. We now report our findings on the dissection and reconstruction projections in the leg nerves of two orthopteran insects, the cockroach (Periplaneta americana) and the grasshopper (Melanoplus bivittatus). Mesothoracic legs of anaesthetized animals were transected at different leg segments. Seven days later, cuticle overlying the mesothoracic ganglion was removed and fixative penetrated the neuropile. The main legs nerves (nerve 5) were cut at its entrance to the coxa and removed with the attached mesothoracic ganglion. After dehydration and embedment, thin and thick cross-sections were cut from the nerve's cut ends. Images of leg nerves of control animals showed a tightly packed, continuous array of axon profiles. In experimental animals, when the most distal leg segments were removed, a discrete area of degeneration was repeatedly found in the loose nerve along its posterior edge. More proximal ablations produced larger areas of degeneration that progressively extended into the anterior half of the nervous system. Overlaying the mesothoracic ganglion showed a posterior-to-anterior layering that generally corresponded to a distal-to-proximal map of the leg. Remarkably similar patterns of degeneration were seen in both cockroaches and grasshoppers.

These experiments indicate that insect peripheral nerves possess a higher level of organization that has previously been recognized. We are currently investigating this arrangement in projections into the central nervous system. Supported by NSF BNS-77-03317.


The methylxanthine phosphodiesterase inhibitors have been shown to increase membrane calcium fluxes and cause the release of calcium from intracellular storage sites. We have investigated the effect of two methylxanthines, caffeine and theophylline, on the slow currents regulating firing frequency in the giant neurons R2 and LPG of Aplysia californica. We have previously demonstrated the calcium dependence of the slow outward K+ current underlying adaptation in these neurons (Zbics & Wilson, Soc. Neurosci. Abstr., Vol. 4, p. 211, 1978). Voltage clamp studies have demonstrated that depolarization of the giant neurons to potentials more positive than -40 mV activates a transient inward current which is followed by a gradually increasing K+ current which mediates adaptation. When the current-voltage relationship of a cell is plotted using current data taken at the time of maximum inward current (0.5-1.0 sec after the voltage step change), a negative resistance region is present at potentials more positive than -40 mV. Removal of sodium or calcium from the external solution depresses or eliminates the inward current and negative resistance region as does application of 1-3 mM lanthanum.

Both caffeine and theophylline (1-10 mM) increase the magnitude of the inward current, steepen the negative resistance region, and increase the development of the slow outward K+ current. Neither the non-methylxanthine phosphodiesterase inhibitor papaverine nor the dibutyryl derivative of cAMP or cGMP enhance the inward transient current or steepen the negative resistance region. These substances are also ineffective in enhancing the development of the slow outward K+ current. The effects of methylxanthines on cell firing in response to stimulation by transmembrane current passage are complex, with enhancement of firing frequency generally occurring. In those neurons whose K+ currents can be manipulated, initial firing of the outward K+ current is increased and the effects are enhanced by removal of outward K+ current. The initial firing current may be enhanced while the frequency of firing after several seconds of stimulation is depressed. It is concluded that methylxanthines increase the inward K+ currents by increasing the magnitude of an inward current carried by sodium and/or calcium with depression of firing occurring when the slow, calcium dependent K+ current is greatly increased.
LIMBIC SYSTEM

Experiments were performed on Mongolian gerbils (Meriones Unguiculatus). The gerbils were exposed to bilateral ischemia by occluding the common carotid arteries. This was found to produce ischemic attacks of varying duration in the Mongolian gerbil. The gerbil is uniquely sensitive to this procedure, perhaps due to the lack of a posterior communicating artery. In an attempt to discern the effects of ischemia on energy metabolism, levels of glucose, ATP, and phosphocreatine were measured in the CA1 and CA3 regions of dorsal hippocampus. The hippocampus was chosen for its well-defined architectonic structure. Further, this allowed a comparison of the effect of ischemia to the well-known effect of hypoxia on Sommer's sector (CA1). Ischemia was produced as follows: both common carotid arteries were loosely ligated with surgical thread while the animal was anesthetized. After recovery from anesthesia the vessels were occluded with Neofix aneurysm clips. Animals were sacrificed by immersion in liquid nitrogen. This procedure freezes the brain at a rate of 2-3 sec/mm, thereby altering the dorsal hippocampal tissue environment sufficiently to stop metabolic activity within 5-10 seconds. After sectioning on a cryostate (-20°C), the tissue was lyophilized and further dissection was performed at room temperature. The samples were weighed on quartz-fiber fishscale balances. Levels of glucose, ATP and phosphocreatine were analyzed using enzymatic assays which take advantage of the fact that NADH fluoresces while NADP+ does not. The data presented describe how the energy metabolism in two regions of the gerbil dorsal hippocampus responds to the metabolic perturbation of ischemic trauma.

886 NEUROGENESIS IN THE RAT HIPPOCAMPAL REGION. Shirley A. Bayer, Dept. of Biol., Purdue Univ., West Lafayette, IN 47907.

Neurogenesis in the rat hippocampal region was examined with 3H-thymidine autoradiography. The rats in the prenatal groups were the offspring of pregnant females given two injections of 3H-thymidine on consecutive days in an overlapping series: embryonic day (E) 13+14, E14+E15, .... E21+E22. The rats in the postnatal (P) groups were injected in a nonoverlapping series: the offspring of pregnant females given two injections of 3H-thymidine on the day of birth (P0) and P1, P2+P3, .... P18+P19. On 60 days of age, the percentage of labelled cells and the proportion of cells added during each day of formation were determined at several anatomical levels within each structure of the hippocampal region (entorhinal cortex, parasubicular, presubicular, subiculum, Ammon's horn, and the dentate gyrus). The neurons in each structure arrive in overlapping, but still significantly different waves: the entorhinal cortex between E15-E17; the para- and presubicular between E16-E19; the subiculum between E16-E18; large cells in the strata oriens, radiculare, lacunosum-moleculare of Ammon's horn between E15-E17; Ammon's horn pyramidal cells between E17-E19; large cells in the dentate hilus and molecular layer between E15-E19. Dentate granule cells begin to originate on E17, and 10% of the population forms after P18. There are three characteristic gradients of formations within each structure: deep cells are generated before superficial cells; cells closer to the rhinal fissure are generated earlier than those lying farther away ("rhinal to dentate" gradient). The first cells formed in the hippocampal regions are the "sandwich gradient" in the entorhinal cortex, Ammon's horn, and the dentate gyrus. There is a "rhinal to dentate" gradient in the ventricles: first, the choroid plexus; second, the subiculum; third, field CA3 of Ammon's horn; fourth, the dentate gyrus. The para- and presubicular are exceptions and form significantly later than the subiculum; CA1 forms significantly later than adjacent CA3 cells. This late neurogenesis may be related to prominent thalamic input to both structures.

887 DISCRIMINATION LEARNING AND REVERSAL FOLLOWING ELECTROLYTIC INJURIES TO RABBIT HIPPOCAMPSUS. Karen E. Ang, David W. Witschel & Ernest W. Kent, Dept. Psychology, University of Illinois at Chicago Circle, Chicago, IL 60680.

Anatomical and electrophysiological studies have demonstrated profound interconnections between the mammillary nuclei of the rabbit and certain limbic structures. At post mortem of this society we (Asin et al., 1976, 1977, 1978) have noted that a number of the behavioral effects of median rhaphe lesions resemble those seen after damage to limbic structures, especially the septum and hippocampus. In the present report we extend our previous findings by describing the effects of median rhaphe lesions on the acquisition and reversal of several food reinforced T-maze discrimination tasks. Similar to what has been reported following hippocampal damage, electrolytic median rhaphe lesions were without effect on the acquisition of a T-maze position habit, but severely impaired its reversal. It is likely that this deficit, in part, reflects damage to serotoninergic elements since a similar, but less pronounced, deficit was obtained following treatment with p-chlorophenylalanine (pCA) (2 x 10mg/kg). When trained on a simultaneous brightness discrimination and eight consecutive reversals, median rhaphe lesioned animals did not differ from controls. A similar dissociation between position and brightness reversal has been reported following septal and hippocampal damage. The acquisition of a successive brightness discrimination was extremely impaired following median raphe damage; reversal was not studied since a number of lesioned animals failed to reach acquisition criterion despite extensive training. This deficit is likely to reflect damage to non-serotonergic elements since pCA altered neither the acquisition nor the reversal of this task. Impaired acquisition of successive discriminations has also been reported following limbic damage.

The current results provide further behavioral evidence for the anatomically derived concept of a limbic-nibra circuit.
MOTOR EFFECTS INDUCED BY INTRA-ACCUMBENS INJECTIONS OF DOPAMINE IN THE SQUIRREL MONKEY. Sherry L. Berg*, Daniel L. Jones, Roy L. Dorris* and Russell E. Bell*. Dept. of Microscopic Anatomy, Baylor College of Dentistry, Dallas, Texas 75246.

Naive male squirrel monkeys, Saimiri sciureus, were used to test drug-induced changes in motor activity. Drugs were infused through chronically implanted cannulae in the nucleus accumbens. Changes in notorioc activity were indexed by computerized analyses of photocell interruptions in the four quadrants of the test cage. Animals were pretreated with RO 4-1284 and then infused with dopamine. The study is an extension of the findings of Dill, R.E., et al., Neurosci. Abst. '78, and is designed to assess the role of the nucleus accumbens in the integration of gross levels of motor activity. (Supported by NIMHCS Grant NS 15020.)


Septal lesions are reported to increase aggression among male hamsters (e.g. Sodetz & Bunell, Physiol. Behav. 5:78, 1970) while preoptic areas lesions decrease it among females (e.g. Hammond & Rowe, Physiol. Behav. 17:507, 1977). It would be of interest to compare these lesions in a single gender. Furthermore, these studies have involved unrestrained encounters in which a lesion's effects on the operated subject's aggressiveness is confounded with its effects on his aggression-eliciting characteristics vis-à-vis his opponent. We report here that anteroventral septal (AVS) and preoptic area (POA) lesions have differential effects on male hamsters' attacks on a standard, non-aggressive, muzzled and analgesic-treated male target hamster.

Twenty-four adult male hamsters selected for moderate preoperative aggressiveness were subjected to radiofrequency AVS or POA lesions or sham surgery. After a two week recovery, subjects were given (a) three 45 min sessions with a standard target, (b) one 45 min session with an estrogen/progesterone primed female and (c) one 45 min session on an activity meter. All tests took place in the subject's home cage with a 48 hr. intersession interval.

A statistically significant difference in attack rate among the groups was largely due to the elevated rate of AVS subjects (mean of 9.6 biting attacks/session) compared to POA subjects (1.1 attacks/session) and sham subjects (1.3 attacks/session). These differences in aggressiveness were confirmed by unrestrained pair encounters: AVS subjects defeated POA subjects in every contest. Twenty-eight per cent, 85% and 100% of the AVS, POA and sham subjects, respectively, copulated with the female. Their respective activity counts were 1501, 368 and 256. Activity counts and attack rates were significantly correlated for AVS subjects but not for the others. In contrast to these differential effects, AVS and POA subjects were similar in their deficiencies in nestbuilding and food-hoarding relative to sham animals.

Our aggression testing technique eliminates the possibility that AVS lesions act solely by altering the aggression-eliciting characteristics of operated hamsters but raise the possibility that attack rate increases are secondary to activity level changes. (Supported by an N.I.H. Guggenheim Foundation Grant)

EFFECTS OF ELECTRODE STIMULATION OF THE SUBSTANTIA INNOMINATA UPON HYPOTHALAMICALLY-ELICITED AGGRESSIVE BEHAVIOR IN THE CAT. C.H.Block*, A. Siegel, H.Edinger. Departments of Physiology and Neurosciences, College of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ 07103.

The substantia innominata (SI) supplies afferents to the lateral hypothalamus, ventral tegmental area, and amygdala. Initiation or modulation of attack behavior can be generated by electrical stimulation of the three brain areas noted above. Accordingly, the present investigation was undertaken in order to determine the possible role of the SI in the control of aggressive responses.

Electrodes for both stimulation and recording were bilaterally implanted in SI and hypothalamus in ten cats. Postoperatively, the effects of stimulation of sites in SI upon either the quiet biting attack (QBA) or affective display of aggression were tested. The effects of SI stimulation were determined by the use of a dual stimulation paradigm which compared stimulation of the hypothalamus alone with stimulation of hypothalamus and SI. The findings suggest a possible differentiation of function within SI. Specifically, stimulation of sites in lateral SI significantly inhibited QBA (< 0.02) while stimulation of more medially placed SI electrodes facilitated the occurrence of QBA (< 0.02). Stimulation of any portion of the SI alone produced no observable behavioral response. These sites that significantly modulated QBA were further studied for their possible effects upon the sensory component of the attack response. It was observed that stimulation of SI sites that suppressed QBA also reduced the extent of the lipline that, when probed, would elicit a jaw opening response, while stimulation through electrodes associated with facilitation of attack produced an opposing effect. The effects upon motor components of QBA were also studied by probing the region of the midline of the lip that consistently elicited a jaw opening response during either single or dual stimulation and the latency for the response was measured. The possibility of a modulatory effect on the motor component of the attack response was suggested as well.

The effects of SI stimulation upon affective display were tested from several sites and sites and sites and sites of stimulation significantly alter the response latency (> 0.1).

Presently, these data imply that the SI differentially modulates the quiet biting form of attack and it appears to do so primarily by acting upon the sensory component of the response mechanism. (Supported by N.I.H. Grant NS 07941-10)
Differential effects of lesions in postero-dorsal septum and dorsomedial frontal cortex on spatial alternation at two inter-trial intervals. G. M. O. Brito and G. J. Thomas. Department of Animal Physiology, University of California, Davis, CA 95616.


Acquisition of hippocampal self-stimulation is facilitated by kindling of the contralateral hippocampus. Kenneth A. Campbell*, and W. W. Milgram. Dept. Psychology, University of Toronto, Scarborough College, West Hill, Ontario, Canada.

We have previously reported that while rats are usually very slow to learn to lever press for hippocampal stimulus, a single session of reinforcement on the day following the TTB increased progressively to reach a maximum in Stage IV. The changes recorded in the DG, CA1 AEPs showed a similar progression of changes in waveform, amplitude and peak latencies associated with the stages of kindling, whereas the AEP recorded in the PPC showed only a slight increase in latency and amplitude. In contrast, lesions in the hippocampal formation produced electrophysically displayed discharges which increased in frequency to reach a maximum in Stage IV or V. The results suggest that interference with septo-hippocampal circuitry temporarily reduces the ability of rats to cope with spatial alternation at short ITI, but at long ITI's they are permanently (at least for 9 sessions) unable to perform above a random level. On the other hand, lesions in postero-dorsal septum, when tested 2 wk postoperatively, dropped to random choices during early testing, but by the 9th session, they recovered to perform near perfectly as did all controls.

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In previous studies we have demonstrated that selective transections of the direct thalamo-cortical fibers after they exit from the posterior commissural fornix produce behavioral changes similar to those reported following large hippocampal or total fornix lesions (Davies and Kent, 1979; Hirs and Davies, 1979). As an extension of this work the effects of damage to these fibers on the acquisition and performance of an operant response sequence was examined (SLT). This task involved learning to press in any one of 4 closely spaced bars in a randomly assigned position in one wall of an operant chamber. Only the first response on any given bar was reinforced. After all 4 bars had been depressed at least once a trial was complete and the levers were retracted to a position outside the chamber as a reset signal to the animals. The number of total and perseverative errors were recorded separately for each trial.

Initial procedures consisted of 14 days of CRF training. After 14 days on the SLT; 10 trials/day. Animals with transections of the subiculo-thalamic fibers (AT) were impaired only on the initial days of SLT making more total errors than normals. On later days the performance of AT animals was not different from operated controls (OC) on either error measure. However, further analysis of the response patterns of both groups revealed that AT animals were adhering to different response strategies than OC animals. Usually brain damaged animals adopt rigid response chains such as response randomly performed. Controls exhibited a much more flexible response pattern.

As an addition of more easily discriminable manipulanda (patterned stimuli on the bars) transiently disrupted performance of both groups. The total number of errors made by all animals was elevated on the first day of this manipulation but quickly returned to baseline levels. Cuin increased the frequency of perseverative errors on the first day of introduction. Thereafter, cues selectively reduced the number of perseverative errors made by AT animals. Taken together these results suggest that AT OC animals learn this task in two entirely different ways.


AT sites in the lateral septum in which electrical stimulation (0.5 - 1.0 sec train of biphasic waves; pulse duration, 0.5 msec; frequency, 100 cps; 2.0 - 14.0 V) produced cardiovascular changes included in the anesthetized rat (Equithesin, .52/cu/10g), a subsequent injection of kainic acid (4 µg in 1.0 µl, injected at 0.2 µl/min) typically resulted in a pronounced tachycardia. Within minutes after the conclusion of the kainic acid administration, sustained myocardial isocytosis is observed bilaterally in forelimbs, jaw, and neck. Equivalent volumes of saline in these locations do not result in these effects. Chemical and electrophysiological stimulation of the caudate nucleus (1.5 mm lateral to the septal sites) produces no observable cardiovascular or muscular responses. The effects of electrical stimulation of the lateral septum are in agreement with the findings of Hollostock (Psychon. Sci., 9:37-38, 1967). The effects of kainic acid and administration to the lateral septum on cardiovascual and electromyographic variables have not been previously reported.

A COMBINED INTRACELLULAR HRP AND GOLGI ANALYSIS OF THE NUCLEUS ACCUMBENS SEPTI. J.F. DeFrance, J.E. Marchand, R.B. Chronister, R.W. Sites and J. Hubbard. Dept. of Neuroanatomy and Neurobiology, Univ. of Texas Medical School, Houston, Texas 77025 and The Dept. of Anatomy, Univ. of South Alabama, Mobile, Alabama 36688.

The nucleus accumbens septi (NAC) has an important parallel limbic structure in standing in relation to both the limbic system and basal ganglia. The cytoarchitectural appearance of the NAC resembles that of a large globus pallidus, intimate connections receive and process large callosal projection via the fimbria (Swanson et al., 1977). Swanson et al. (1977) have described a lateral fornix projection via the stria terminalis (ST) (DeOlmos, 1971). The present study was undertaken to determine the morphological appearance of the NAC septi neurons receiving monosynaptic IFim and ST input.

Rabbits were acutely prepared under urethane anesthesia. The cornea and corpus callosum were removed for the exposure. A 2 x 1 mm electrode was lowered through the position of the microstimulation and recording electrodes. The recording electrodes were filled with 10% HRP (4X-10X) in Tris buffer plus UM HCl. Extracellular unitary responses were recorded following IFim and ST stimulation and characterized with power and paired-stimulus testing. The cells were then injected with HRP. For the correlograph analysis, NAC septi tissue was prepared according to the Rapid Golgi and Golgi-Kopach techniques. Monosynaptically excitatory input via the IFim and ST is largely restricted to dorsal-caudal halves of the NAC. From the HRP analyses, there are two distinctly different cell types which are the recipient of both inputs. Firstly, there is a small (6-15 µm) cell with spiny dendrites. The dendritic pattern is spherical with the dendrites themselves being delicate. The cell body is free from spiny appearance commencing on the primary dendrites. Second, there is a larger (15-25 µm) cell in which the dendrites are essentially spine-free. The dendritic pattern is again spherical, but the dendrites are thicker. These appear to reside more medially and ventrally in the NAcS.

The Golgi analysis confirmed that these two cell types indeed exist in the NAC. It further shows that the small spiny neurons are the dominant cell type being scattered throughout the nucleus.

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References


Rats with rewarding electrodes in the lateral posterior hypothalamus were trained to an avoidance paradigm. A brain stimulation delivered through the rewarding electrode is used as conditioned stimulus (CS). Test for generalization to other brain stimula-tions (substitution of another CS or SD) were the critical values of the electrical stimulus parameters (i.e. intensity, frequency, pulse duration, and train duration).

The results showed that two types of test parameters were obtained. A two-way gradient was obtained for pulse frequency and for train duration while intensity and pulse width modifications produced a one-way gradient. These data suggest that the generalization and discrimination phenomena observed are not subject to the percentage of avoidance responses during the generalization tests, and the rewarding intensity, as measured by the scaling of the avoidance schedule. These data have been analyzed by the Hypothesis testing. In all cases, the data fitted a power function. It appears that the perception of the rewarding value through a reinforcing brain area follows the same linearity as with human conditioning tests. It should be noted that the value of the exponents does not deviate much from unit. These data thus indicate that the calculated exponents are, a.e., in other words, in the rewarding intensity elicited by brain stimulations is accompanied by the same relative change in avoidance responses. This may serve to suggest that, in the brain, no noise occurs between the transformation from the input, i.e. the intensity of the brain stimulation, and the output, i.e. the reinforcing sensation as estimated by the conditioned test. Our experiments indicate that the degree of reward is a perceived dimension and that it is being processed according to the psychophysiological laws.

In rodents, active investigation is characterized by vigorous sniffing and the presence of hippocampal rhythm slow wave activity (RSA) in the hippocampus (e.g. Meibach and Siegel, 1977, Brain Research, 124, 197-224). These studies have been observed to entail while the animal appeared to be investigating an odor. Quantitative analysis of this phenomenon would be aided by development of methods which would enable the odor stimulation on repeated trials and under conditions in which the chemical compositions and concentrations of the stimulus can be well regulated. For this purpose, a paradigm involving multiple reversal odor discrimination was devised and the relationship between hippocampal RSA and sniffing during reversal learning was characterized with retrospective computer analyses based on the Fast Fourier Transform (FFT). Rats were trained in a shielded arena with a port in one wall behind which clean air or odors, generated by a flow rate diluter, were passed. Proximity to the odor was recorded with a photocell at the port. An animal could initiate a trial by crossing another photocell located 36 cm from the port, and thereafter allowing one of two odors. For an S+ odor stimulus the rat could hold its nose in the port continuously for 2 seconds in order to obtain a reward which was delivered at a level below the port. No reward was given for S- odor trials, and animals learned not to hold their nose in the port on such trials. Sniffing was monitored with a thermocouple placed in the nasal cavity via an implanted stainless steel activity which was connected to the dorsal hippocampus with implanted bipolar electrodes.

Rats rapidly acquired a discrimination of phenethyl alcohol versus geraniol, and this readily acquired distinction was maintained for the ongoing hippocampal RSA. Moreover, the FFT analyses verified that the rats timed these sniffs with a preferred phase relationship to individual cycles of the hippocampal RSA. The entrainment of sniffing often occurred on every trial during criterion-level performance, but was particularly prominent soon after the onsets of the response that were not correlated with the assessment of an odor's behavioral significance.

(Supported by NINCDS grant NS 12344 and NSF grant BNS77-24405.)
INTRAHIPPOCAMPAL INFUSION OF NOREPINEPHRINE OR CARBACHOL INCREASES LOCOMOTOR ACTIVITY IN RATS. Charles Flicker* and Mark A. Gaynor. Dept. Psychiatry and Neurosciences, Sch. Med., UCIrvine, CA 92668.

Intracerebroventricular infusion of either norepinephrine (NE) or carbachol (CH) into the hippocampus has been reported to result in increased spontaneous locomotor activity in rats. One site at which this response may be mediated is the hippocampus. A NE input to the hippocampus has been demonstrated via a noradrenergic bundle originating in the nucleus locus coeruleus. The septohippocampal pathway is an established cholinergic input arising from the basal forebrain septal region. This is the putative cholinergic bundle. The presumed effects of transmitter release from these two projections by the direct infusion of appropriate agonists into the hippocampus has been examined in an experimental situation.

Male Sprague Dawley rats (350-400g) were chronically implanted under Nembutal anesthesia with guide cannulas aimed bilaterally for the rostro-caudal hippocampus. After a week of recovery from surgery the animals were connected to sham infusion lines and acclimated to the experimental chamber for 30 minutes. The experimental chamber consisted of a 12" x 24" x 15" holeboard box with 11 holes in the floor and walls.

A 30-gauge needle at the end of each infusion line was attached to each cannula. The needle's tip protruded 1.5 mm beyond the cannula's. Animals were then placed in the holeboard where - in addition to visual observation of behavior - locomotor activity and hole poking were monitored as well. Measurements were taken by electrical contacts with a steel wall plate, monitored by computer.

After a 30 minute pre-infusion period, the animals received a 40 minute infusion of 1,500 microliter of saline or of NE or CH (3 μg/μl dissolved in saline) or physiological saline at a rate of 25 nanoliters per minute. In all treatments, the infusion occurred at randomized intervals of 5-7 days. Some animals were pre-treated with systemic injection of a muscimol antagonist, either atropine sulfate or methyl atropine (i.p. 1 μg/kg). The animals were then retested as above.

The mechanical disruption caused by the insertion of a 30-gauge needle or the slow infusion of saline into the hippocampus of the rat frequently induced "wet dog" shakes which are commonly associated with electrical seizure activity in the structure. Despite these symptoms of abnormal baseline activity, infusion of either NE or CH into the hippocampus produced a transient increase in spontaneous locomotor activity as compared to saline controls. Results obtained with the various treatments are compatible with the hypothesis that this behavioral activation is mediated by a noradrenergic-cholinergic interaction at the level of the hippocampus.

The goal of our work has been to characterize the neuronal circuitry of the entorhinal area of the rat and their role in the formation of spatial codes. In this paper we described the results obtained applied three different techniques: horseradish peroxidase, evoked potentials and unit activity.

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"Spatials" have been identified in micro-electrode recordings from dorsal hippocampus of rats. Such units fire at their maximum rates only when rats are in specific regions of the recording space, called the units' "fields," and seem to be independent of details of the animals' behavior.

Spinal units were recorded from rats that had been trained to perform a spatial alternation task in an enclosed automated T-maze. Observations were made during and after various changes made within the recording environment. Spatial changes included covering parts of the maze with striped cloth, varying the level and location of light sources and covering the rats' eyes with a rubber mask. Defactory tests included thorough cleaning of the apparatus, and introduction of strong odors (cologne, acetone) or other rats into the field region. Auditory stimuli were changed by placing a speaker which emitted sharp clicks at various locations around the maze, and by inserting baffles or opening the top of the maze to change its acoustical properties. The rat's running path was altered by introducing walls and bars into the maze. Finally rats were spun at 70 rpm in the start box between trials to produce spurious vestibular cues.

Spatial firing was eliminated in 3 of 12 cases by visual alterations and in 6 out of 12 cases by changes in running path. No other changes were found. Results indicate that hippocampal spatial firing is based on multi-modal sensory cues. In order to explore this aspect of spatial firing in more detail, groups of rats were selectively deprived of either vision (by masking or enucleation), hearing (by chronic administration of neomycin antibiotic), olfaction (by intranasal ZnSO4), or vibrissal input (by shaving). Spatial firing was found reliably in rats from each group, the four groups showing at least 2 cases of each stimulus in 2 different rats in each group. Mortality and treatment effectiveness varied between groups, making comparisons using large numbers of subjects unreliable. Further observations indicate that there are no differences between spatial firing found in any of the sensory-deprived rats and that found in normal animals. Results show that spatial firing is not, in general, a purely sensory response via any one of the above modalities.

912 FREQUENCY POTENTIATION OF RESPONSES TO FIMBRIAL STIMULATION IN RABBIT MEDIAL MEPPHORYC NUCLEUS. John J. Hubbard* and Jon F. DeFrance. Dept. of Neurochemistry & Anatomy, University of Texas Medical School, Houston, TX 77025.

Field potentials recorded in the medial preoptic nucleus (MPO) of urethane anesthetized rabbits following ipsilateral fimbrial stimulation consisted of an initial spike-like negativity (latency to peak 2 msec) followed by a negative wave (latency to peak 10 msec). The position of recording and stimulating sites was confirmed by passage of fast green dye from the electrodes and later examination of fixed tissue in 12 μm frozen sections. The field was maximal in the MPO about 1 mm below the anterior commissure and 100 μm lateral to the midline.

All components of the field had similar thresholds and recording of single units suggested that the field was explicable by an entering volley, the excitation of MPO cells and the recurrent inhibition of these cells.

The negative and positive waves increased 2-4 times in amplitude 2-12 Hz in response, which was not seen at 6-8 Hz, the natural output frequency of the hippocampus. There was 1-3 sec of post-tetanic potentiation.


Our initial cytoarchitectonic studies of the limbic lobe (Lol) in the cetacean brain have concentrated on the pericellosal (superior) limbic formations which are sharply and constantly delineated on the medial surface of the hemisphere by the collosal sulcus (SCS) and by the cetacean homologue of the sulcus cinguli we term the limbic cleft (CL). Several significant dichotomies are evident as compared to terrestrial mammals including man.

The cell architecture of the limbic cortex nowhere shows sharp transitions. The only abrupt and sudden changes as it is followed both outward from the limbic bank of SCS (pericellosal area, PF) over the free surface of the Lol, limbic area proper, AMC, the limbic bank of the CL, and also anteriorly and anteroposteriorly from the subgenual region to the splenium of the corpus callosum. In the cetacean brain the internal granular layer (layer IV) is weakly developed throughout the neocortex and is totally absent from the posterior limbic region in sharp contrast to the territorial pattern. Anterior limbic sectors are, overall, paucicellular as compared to posterior limbic areas. Many of the cells present, however, are larger than found posteriorly. Cell density increases very gradually towards the posterior limbic region, the cells that appear being predominantly small in size. This increased cellularity in supragranular layers posteriorly results in the boundary between layers II and III being less distinct than anteriorly. The presence of these small cells in layer V and the tendency for cells to organize into columns, a pattern in contrast to the terrestrial patterns indicate that the cetacean limbic cortex reflects a special organization not found in land mammals.

From SCC to CL the cell patterns of anterior and posterior limbic sectors become more definitive.

In summary, significant cytoarchitectonic variations from territorial patterns indicate that the cetacean limbic cortex reflects a special organization not found in land mammals. However, despite such a claim there still seems to be laminated limbic cortex and areal differentiation into subsectors (Supported by NSF Grant # BNS 78-08660.)


The anteroventral cortex (AVC) supports intracranial self-stimulation (ICSS) and is a site of orthodromic and antidromic activation from stimulation of other ICSS sites. The purpose of this study was to determine, in the unrestrained rat, the activity patterns of AVC neurons during ICSS, motivated behavior related to drinking, and the response to various sensory stimuli.

Rats were tested in a cylindrical chamber, the walls of which contained three holes 30 degrees apart. Contact with a bar presented through any of these holes produced 50Hz stimulation of the ventral tegmental area which continued until the animal contacted a 10x15 cm plate on the wall opposite the hole. The water-deprived rat was provided 0.1% saccharine through a tube presented also via these holes. Tests for sensory responsiveness included the presentation of a moving black-white grid. Single units were typically held for more than one hour.

A total of 51 single units have been studied in three rats. The most common well-defined class of neurons was related to orienting behavior (n=24). They increased in activity when the rat actively investigated any of several classes of stimuli. These units lacked any of the normal ICSS trains or during drinking, but they tended to show an increase at the behavioral response which produced stimulation onset. Activity in another seven units was related to head movements (six units increased while one decreased). Activity in three units was associated with sniffing, and another two units increased activity when the rat oriented to, or was situated at the locus of the offset grid. The largest class of neurons (n=19) showed no discernible pattern consistently correlated with any of the stimuli or behavior studied. A principal axis of rotation (x-pos) seemed to code aversive and rewarding states by increases or decreases respectively in rates of activity. Increases were seen when the animal showed distress to tactile stimulation, noxious odors, or the experimenter's hand. Decreases were seen when the rat oriented to conspecific urine, and to the holes in the chamber wall. Removal of the experimenter's hand was followed by a decrease in activity. These patterns of activity support the tentative suggestion that the AVC might be a site at which motivational information influences systems which participate in the control of orienting behavior.

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We have developed a mathematical model of hippocampal function that is intended to describe the interactions of input activity with the activity of various intrahippocampal populations of neurons. The pools of neurons that are assumed to be functionally homogeneous include the septal and entorhinal sources of input, and the granule pyramids and basket cells. The model is bilaterally symmetrical, with activity of each homogeneous population in each hemisphere ordered nonlinearly with a differential equation. Parameters are simulated, with activity defined in relative units ranging from 0 to 1. The 26 equation pools in each hemisphere are solved simultaneously by computer to produce plots of the time course of activity changes in each of the populations.

Each neuronal pool has its own specific equation of the form that is based on its known functional connection, but the general form of the equation is:

\[
dX_i/dt = (X_i/X_{max})(X_{max}-X_i)(IE_{x_i} + \sum_{j} EE_{ij}X_j + I N_{N_i} - X_i)
\]

where \(X_i\) = relative activity in any given pool, \(X_{max}\) = maximum possible activity in any given pool, \(IE\) = internal (recurrent) excitation within a pool, \(EE\) = external excitation from each of the various inputs to the pool, \(IN\) = external inhibition from inputs to the pool, \(N_i\) = nonneural inhibition, and \(D\) = a decay constant (i.e., rate at which activity would decay in a given population if input ceased).

The simulations performed thus far seem to be useful in evaluating the population dynamics that might underlie such hippocampal processes as the electroencephalographic "theta" rhythm. Solutions to certain of the equations can be made by appropriate parameter assignment to oscillate at different frequencies or to oscillate at a non-oscillating steady state of activity.

**Relationship between the Limbic Theta Rhythm and Neuronal Activity in the Olfactory Bulb**

The present study examined the temporal relationship between the limbic theta rhythm, monitored as rhythmic slow wave activity (RSA) in the dorsal hippocampus, and the slow wave and spontaneous single unit activity recorded simultaneously in the main olfactory bulb (MOB) of adult male hamsters. The olfactory bulb and granular and pyramidal cells are synchronized with hilar and granule cells. Postsynaptic potentials (PSPs) from perforant path (PP) could arise from several mechanisms which are not definitely identified. They may be due to 1) dendritic spikes; 2) electrotonic coupling; and 3) axonal spikes. The present results suggest that these or related centrifugal projections may interact with local circuits in the MOB to modulate the temporal firing patterns.

**Morphology and Electrophysiology of Dentine Granule Cells in the Chronic Implant Rat**

The hippocampal CA1 region in the chronic implanted rat: A study of anesthetic action and behavioral states

The hippocampal CA1 region in the chronic implanted rat: A study of anesthetic action and behavioral states

W. B. Klemm, N. N. Naugle, and G. M. Harmwell. Dept. Biology, Texas A&M University, College Station, Tex. 77843.

We have developed a mathematical model of hippocampal function that is intended to describe the interactions of input activity with the activity of various intrahippocampal populations of neurons. The pools of neurons that are assumed to be functionally homogeneous include the septal and entorhinal sources of input, and the granule pyramids and basket cells. The model is bilaterally symmetrical, with activity of each homogeneous population in each hemisphere ordered nonlinearly with a differential equation. Parameters are simulated, with activity defined in relative units ranging from 0 to 1. The 26 equation pools in each hemisphere are solved simultaneously by computer to produce plots of the time course of activity changes in each of the populations.

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(Supported by NSF grant BNS78-06248 and NINCDS grant NS 12344.)

The local intracerebral injection of kainic acid, an analogue of glutamate, reportedly destroys intrinsic neuronal cell bodies but not afferent fibers. In the present study, kainic acid or phosphate buffer alone were injected into the amygdala-piriform cortex area of the rat. The effects on the cholinergic enzymatic, choline acetyltransferase (CAT) and acetylcholinesterase (AChE), and the binding of the muscarinic ligand, 3H-quinuclidyl benzilate (QNB), and the nicotinic ligand, 125I-alpha bungarotoxin (aBuTX), were investigated. Twenty-four hr following the injection of kainic acid, CAT activity in the amygdala-piriform cortex area was reduced to 65% of the control. The reduction in CAT activity was time-dependent with the maximal reduction occurring at 5 days post-injection of kainic acid. Kainic acid also reduced the AChE activity by 17% at 24 hr and remained unchanged. At 24 hr, the binding of QNB and aBuTX was also unchanged. By 72 hr, the number of QNB and aBuTX binding sites were reduced to approximately 65% of the control. The number of sites fell to approximately 45-50% of the control by 5 days and remained at this level. In addition, at 72 hr the binding of QNB and aBuTX in the amygdala-piriform cortex area contralateral to the kainic acid injection was reduced to 65% of that of the same area in the control rats. The binding of QNB 6 hr and 24 hr post-injection to the injection site remained at 85% of the control. However, binding of aBuTX in the area contralateral to the kainic acid injection site fell to 60% of the control by 2 days and remained unchanged. The results indicate that the loss of QNB and aBuTX sites after kainic acid injection approximates the loss of the same neurones, also with a considerable variability of latency, 15-20 sec.

In addition to the excitatory influence dorsal ST and lateral fimbria stimulation depressed the neuronal localization of the muscarinic and nicotinic receptor sites in the amygdala-piriform cortex area, and further suggested the existence of reciprocal monosynaptic connections between these two areas.

References

Previous horseradish peroxidase (HRP) studies of the subcortical afferent connections in the cat (Mohler, et al., 1976, Anat. Rec. 190:477, 1978) and in the rat (Pretorius, et al., Anat. Rec. 193:657, 1979) confirmed the presence of some amygdalopetal cells described in the nucleus of the tractus solitarius and a large number of cells from the parabrachial nuclei at pontine levels in the rat (Ricardo and Koh, Brain Res. 153:1-26, 1978). In addition to these cells, HRP injections into various parts of the amygdala in monkeys and rats also labelled cells in the dorsal and medial (Bechtever and linearis) raphe nuclei in the midbrain.

HRP injections of amygdale of 18 cats (3.0 - 0.5 ul, 50% solution, 24-52 hr survival, 1.5% glutaraldehyde - 0.5% paraformaldehyde / pH 7.4, DAB or HCN substrate) demonstrate comparable patterns of HRP-positive (HRP+) cells at pontine and mesencephalic levels. Large numbers of HRP+ cells delineate from the medial geniculate body the feline homologue of the simian and rodent peripennicular nuclei.

In the hypothalamus, varying populations of HRP+ cells appear most consistently in the dorsomedial nucleus and in the lateral hypothalamic area at tuberal and mamillary levels. Labelled to cells appear throughout the ventromedial nucleus only in the most medial amygdala injections. The most imposing pattern of amygdalopetal cell labelling appears in certain ipsilateral subdivisions of the midline nuclei of the dorsal thalamus, namely; the paraventricularis (PA) and a cell group interposed perpendicularly between PA and the nuc. centrales medialis (Com), the latter of which may also send axons to the amygdala. Com, however, appears to project chiefly to the claustrum or adjacent cortical areas (Mehler and Mohler, In preparation). Small HRP injections involving only the dorsal part of the amygdala fail to label PA but uniquely label nuc. subpara- hamarenalis, fimbria or accessory labels cells in the bed nucleus of the stria terminals not seen even in large ventral or lateral quadrant amygdala injections.

Although amygdalopetal labelled cells have been found to exist in the medial part of the nuc. medialis dorsalis in the rat (Pretorius, et al., 1979) there is no evidence, to date, for such connections in either the cats of the present series or in the monkey previously studied (Mohler, 1979, In press). Supported by: NASA Task 199-05-02-07.


Estimates of coherence functions are used to investigate the relationships between signals in two areas of the brain. Some of these signals have been recorded from frontal, occipital and temporal cortex, amygdala, septal and accumbens nuclei. Recordings are made with cats using hardwire and radiotelemetry. This permitted analysis of electrical activity of the brain during free moving and limited motor behavior states.

The coherence of a signal, as 40 Hz rhythm and sensorimotor rhythm (12-14 Hz) (OBM) is a function of frequency. At each frequency of the power spectrum the coherence function has a value between zero to one which gives a measure of correlation or phase locking of the signal in two areas in the brain.

The coherence estimates of 40 Hz, SMR and theta rhythm are made during certain behavioral states, and during the action of central nervous stimulants and depressants. During action of depressants there is a decrease in coherence in the 15 Hz to 40 Hz frequency range. There is a decrease of coherence first between areas of the cerebral cortex followed by cortex and amygdala. In the 1 Hz to 5 Hz range, signals between the cortex and amygdala showed coherence levels higher than those between two areas of the cortex.

Central nervous stimulants, the amphetamines, increased coherence in the 30 Hz to 50 Hz range between cortical areas and cortex and subcortical nuclei. In the 1 Hz to 5 Hz range there was an increase in coherence whether the data came from two cortical areas or a cortical and subcortical area. There is a shift of increased coherence in signals in the 40 Hz to 50 Hz range and highest coherence levels. These findings were more evident in the cerebral cortex and amygdala data.

PLACE UNITS ARE DIFFERENTIALLY DISTRIBUTED WITHIN HIPPOCAMPAL FIELDS IN THE RAT. Virginia H. Miller and Phillip J. Best. Dept. of Psychol., Univ. of Va., Charlottesville, Va. 22901.

Correlation between hippocampal unit activity and an animal's location in space has been reported during a variety of spatial discriminations in rats (Miller, et al., Soc. Neurosci. Abstr. 2: 10, 1976). Previous reports have shown a significant change in firing rate dependent only on an animal's location in space and independent of the animal's activity in that location as well as called place units. Manipulations such as maze rotation which do not disrupt the animal's spatial task performance, do not disrupt place unit integrity. Further, if spatial task performance is debilitating by lesions of hippocampal connections, place unit integrity is also disrupted.

The flow of information through the hippocampus proceeds from the entorhinal cortex to the dentate gyrus and thence to CA1 and CA3. The present study addresses the questions: What is the relative distribution of place units within the hippocampal fields and dentate gyrus, and do lesions of hippocampal connections differentially affect the integrity of place units within each field?

Rats were trained to continuously traverse a radial 8-arm maze for food reward. Hippocampal unit activity was recorded and compared among 3 groups: control (C), no lesion; fornix lesion (FL) and bilateral entorhinal lesion (EL). All of the 18 units recorded in the regio-superior (RS) from the C group were place units. That is, firing rate significantly different from that of other units (P<0.05) as the animal traversed the radial 8-arm maze. Upon 90°rotation of the maze, all units in the C group persisted with increased activity in the original directional orientation. Either lesion, FL or EL, significantly reduced the number and degree of persistence of place units in the RS. In the FL group, 22 of 29 units were place units, 10 of 22 persisted; in the EL group, 10 of 19 units were place units, 10 of 10 persisted.

Fewer place units were found in the regio-inferior (RI) than in the regio-superior in all groups. Only 3 of 8 in the C group, 1 of 7 in the FL group and 4 of 7 in the EL group were place units in the RI. The degree of persistence during maze rotation was not different between the regio-inferior and superior within groups.

The reduction in number of place units in both RS and RI following lesion of hippocampal connections suggests that convergence of input is necessary for the establishment of a unit's place field. The differential distribution of place units within the hippocampal fields has implications as to the sequence of processing of spatial information (Supported by grants NSF #5671854 and NIMH #61678 to PBJ).

HIPPOCAMPAL INFLUENCES ON AMYGDALA UNITS IN THE AWAKE MONKEY. Frederic Morrison and Charles Poletti. Massachusetts General Hospital, Boston, MA 02114.

Amygdala extracellular unit activity in the awake, restrained squirrel monkey was studied in response to hippocampal electrical stimulation. Hippocampal volleys elicited responses in 20% (96) of 476 units tested in three monkeys. The spontaneous firing rate, response latency, and percentage of nonresonant responses were not different in either the rostral, central, or posterior or the contralateral stimulation. Lateral anterior hippocampal stimulation was more effective than posterior or contralateral stimulation. Hippocampal influence was topographically organized. In the six areas with more than 25 tested units, the basomedial nucleus has the highest percentage of responsive units (39%), followed by the accessory basolateral (32%), central (22%), basomedial (18%), and lateral (5%) nuclei, and the anterior amygdala area (4%). Initial excitation (E) was more prevalent than initial inhibition (I) in the central (90% E vs. 10% I) and basomedial (82% E vs. 18% I) nuclei; but initial inhibition was more common in basolateral (37% E vs. 63% I) and accessory basolateral (33% E vs. 67% I) nuclei. The mean response latency was 30.8 msec, ranging from 12-130 msec.

The basomedial nucleus appears to receive the most potent hippocampal influence with the highest percentage of responses to single and multiple shocks and smallest mean latency. In this nucleus there was a preponderance of short latency (12-20 msec), brief (less than 20msec duration) excitatory responses with little variability in latency to single shock stimulation; 16% of tested units in basomedial nucleus had these characteristics compared to only 1% of units in other amygdala nuclei. These response characteristics are consistent with a hypothesized relay of nonofornix hippocampal influences on basal forebrain and hypothalamus via the basomedial nucleus (Poletti and Suzuki, Soc. Neurosci. Abstr. 3: 203, 1977).
929 CONDITIONING OF HIPPOCAMPAL THETA ACTIVITY IN AWAKE, FREE-RANGING CATS. Arden V. Nelson*, William J. Jackson and June Kearns*. It has been shown that theta conditioning of Flaxedillyed animals can be readily accomplished (Black, Am. Sc., 1971, 59:236-253; Glazer, CZEP, 1974, 5:267-273) but little work has been done with animals in their normal awake state. In the present study an attempt was made to condition the unique 4-7 Hz slow wave activity that is associated with the dorsal hippocampus in awake, freeranging cats. We used two different methods to detect the occurrence of theta activity. One method calculated the ratio of 4-7 Hz filtered activity to broadband filtered (0.1-10 Hz) activity, and if the ratio was greater than the target value, a reward was given. The other method measured the wavelength and amplitude of the hippocampal theta activity and if a fixed number of waves met the wavelength and amplitude criterion, a reward was given. The rewards for both groups was electronic stimulation to a pleasure nucleus, which was located in the midbrain.

In other experiments, HRP was injected into the hippocampus. When the injection remained confined to the anterior insula, HRP-positive neurons were observed in several amygdaloid nuclei as a result of the anterior commissure. On the other hand, injections which also involved more caudal parts of the insula resulted in the rostral insula labeling restricted to its medial aspect while more caudal injections resulted in additional labeling more laterally in the same nucleus.

Even though the enzyme and amino acid injections in most cases spread into parts of the claustrum, the extent of this involvement was quite minor. In conclusion, the data would indicate that the insular cortex and amygdala in the rhesus monkey have reciprocal connections that are topographically organized.

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930 SELECTIVE DISTRIBUTION OF PROJECTIONS FROM AMYGDALA TO PREFRONTAL CORTEX IN RHESUS MONKEY. Linda J. Porrino* and Patricia S. Goldman. Sec. Devel. Neurolab., NIMH, Bethesda, MD 20205.

The projections of the amygdala to prefrontal association cortex in rhesus monkeys were studied using horseradish peroxidase histochemistry and autoradiography for tracing neural connections. Microquantities of HRP were injected into one of the five following subdivisions of the amygdala: the lateral nucleus, the ventral nucleus, the central nucleus, the central-medial nucleus, and the basal nucleus. The projections of the amygdala to the superior frontal gyrus, the inferior frontal gyrus, and the orbitofrontal cortex were examined in detail.

Following HRP injections into the superior prefrontal cortex, the amygdala projects to the overlying cortical areas, including the inferior frontal gyrus and the orbitofrontal cortex. The projection to the inferior frontal gyrus is more extensive than that to the orbitofrontal cortex. The projections to the orbitofrontal cortex are most extensive in the inferior frontal gyrus and the orbitofrontal cortex. The projections to the inferior frontal gyrus are most extensive in the inferior frontal gyrus and the orbitofrontal cortex. The projections to the orbitofrontal cortex are most extensive in the inferior frontal gyrus and the orbitofrontal cortex.


The potent convulsant, kainic acid (KA), readily destroys rat hippocampal neurons when injected locally but not systemically. In several regions of brain the neurotoxic action of KA depends on the integrity of excitatory inputs which are thought to be glutamatergic. The target of KA is the N-methyl-D-aspartate (NMDA) receptor. This receptor mediates a large and sustained release of glutamate in response to KA. KA either intraventricularly (3.75 nmol) or locally (2.34 nmol) over a 30-min period. At the doses used in this study, intraventricular KA destroys only the CA3-CA4 cells, whereas locally injected KA destroys the majority of each neuronal type in the hippocampal formation.

Both destruction of hippocampal mossy fibers with colchicine and transection of these fibers markedly attenuated or abolished the subsequent target-destructive effects of intraventricular KA toward CA3 cells. These pretreatments conferred no protection against KA injected locally. Conversely, removal of projections from the entorhinal cortex protected dentate granule cells to a somewhat lesser extent, hippocampal pyramidal cells from destruction by locally injected KA, but little affected the hippocampal toxicity of intraventricular KA. A commissioning approach did not greatly change the hippocampal lesion made by either route of administration. The entorhinal projection to dentate granule cells is probably glutamatergic, and some of the commissural fibers may be also, but the mossy fibers probably are not. Intraventricularly and locally injected KA destroyed somewhat different populations of neurons outside the hippocampal formation. All three types of dentation in KA-treated hippocampi had a common component.

These results emphasize the dependence of KA neurotoxicity on excitatory circuitry, in accordance with the idea that general- and specific-glutamatergic activity play a distinct and possibly differential role in the etiology of KA-induced neuronal degeneration. The critical excitatory pathways probably need not be glutamatergic. Finally, the neurotoxicity of KA to different limbic structures involves somewhat different mechanisms. (Supported by NSF grant BNS 73-13061).

Morphine, methadone enkephalin and other opioid peptides were investigated with respect to their influence on hippocampal neural activity. Field potentials and spontaneous cellular activity were studied following the addition of morphine sulfate, methadone enkephalin or [D-Ala2, Met-enkephalin to the bathing media of rat hippocampal slices maintained in vitro. Recordings from the CA1 molecular and stratum pyramidale hippocampal regions were recorded using a micropipette (filled with NaCl 2M) and 2M NaCl (5 µg/µl) as not to provoke the prolonged depression previously described in the basal forebrain.

These results indicate that if the perifornical path indeed releases glutamate, it is clear that this is confirmed by different from other glutamnergic terminals. One cannot exclude the possibility that baclofen blocks transmission by another mechanism.


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OPIOID PEPTIDES AND CELLULAR FUNCTION IN THE HIPPOCAMPAL SLICE. J.P. Potter, M. Halpern, and D.A. Prince (SPON: J.G. McCormick). Dept. of Neurobiology, University of California, San Diego, La Jolla, CA 92037.

The opioid peptides are potent inhibitors of cellular activity in the hippocampal slice. The effects of the opioid peptides on the action potentials and synaptic potentials were measured using extracellular recording techniques.

These results indicate that specific and nonspecific influences of opioids may be responsible for the excitatory effects of these agents on hippocampal tissue.
935 EFFECT OF RAPHE STIMULATION ON GRANULE CELL ACTIVITY IN THE HIPPOCAMPAL DENTATE GYRUS. Robert S. Sloviter and John D. Connor. Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey PA 17033.

Neurons in the entorhinal cortex innervate (via the perforant path) the dendrites of granule cells in the hippocampal dentate gyrus. Stimulation of this pathway evokes a field potential which reflects hippocampal cell dendrites. Superimposed on this potential is a "population spike" which represents action potentials generated simultaneously in many granule cells. The amplitude of the spike is proportional to the number of granule cells firing (Lømo, 1971).

During stimulation of the perforant path, coincident stimuli to the median raphe nuclei cause a rapid increase in the granule spike amplitude (often 7-10 fold). This response is highly reproducible and site specific (n = 40). Injection of 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), a serotonin (5-HT) receptor agonist, mimicked the effect of raphe stimulation, but only at parameters of entorhinal stimulation where raphe stimuli were effective (n = 8).

Anatomical evidence (Moore & Nalbarg, 1975) suggests that 5-HT neurons innervate basket cells which lie in the hilus of the dentate gyrus. These basket cells are believed to be responsible for a γ-aminobutyric acid (GABA) mediated recurrent inhibition of granule cells. Intravenous injection of bicuculline and picrotoxin (GABA antagonists) also increased the spike amplitude at all perforant path stimulus parameters (n = 6). Strychnine was without effect (n = 6). A number of cells with firing characteristics expected of these inhibitory interneurons i.e. high frequency and long duration, have been recorded in the hilar region. Efforts are underway to determine the effects of raphe stimulation on the firing characteristics of these cells and granule cells.

While single unit studies show mainly inhibitory effects of raphe stimulation (e.g., Segal, 1975), our experiments suggest that the net effect of 5-HT released in the dentate gyrus under these design conditions, is excitatory. Although not proven, the available evidence favors an inhibitory action of 5-HT on inhibitory interneurons.

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936 THE EFFECT OF MORPHINE ON THE EXCITABILITY OF HIPPOCAMPAL PYRAMIDAL CELLS. Stanley, J.C., DeFrance, J.P., Taber, K. and Dafny, M. Dept. of Neurobiology and Anatomy, Univ. Texas Medical School, Houston, TX 77025.

The hippocampal formation (HF) is a major component of the brain's limbic system, it is thought to mediate part of the opiate response (Simmon and Hiller, 1978). Therefore, the effect of morphine with respect to the excitability of hippocampal pyramidal cells was studied in rabbits acutely prepared under urethane anesthesia.

Glass microelectrodes configured in either a 3-barrel or a 4-barrel array were used for recording and the iontophoresis of drugs. The drug ejection barrels contained either morphine sulfate (0.1M, pH 6.5) or naloxone hydrochloride (0.1M, pH 6.3) mixed in de-ionized H2O.

The analysis of the morphine effect was based upon HF field potential changes. Monosynaptically activated field potentials were recorded in CA1 following microstimulation of the contralateral field CA3.

The results indicate that: (1) morphine has a potent excitatory effect upon hippocampal pyramidal neurons, (2) the most effective layer is within the pyramidal cell body stratum, (3) the excitatory effect is readily reversed by naloxone, and (4) there appears to be a "super-sensitivity" phenomenon.

The fact that at subthreshold stimulus intensities for the population spikes, the positive component is diminished by morphine with a subsequent appearance of the population spike is consistent with the conclusion of Siggins et al. (1978) that morphine enhanced pyramidal cell excitability by the inhibition of interneurons.

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References

937 EVIDENCE FOR THE ACTION OF DIAZEPAM ON BOTH HIPPOCAMPAL PYRAMIDAL CELLS AND INHIBITORY INTERNEURONS. K.H. Taber, J.F. DeFrance and J.C. Stanley. Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77025.

Diazepam is known to alter the excitability of hippocampal neurons. We have found that diazepam, administered intrathecally, suppressed the post-tetanic potentiation (PTP) of hippocampal pyramidal cells, while at the same time enhancing tetanic potentiation (TP). This study was undertaken to clarify the mechanism of the enhanced TP following diazepam administration. Animals were initially prepared under urethane anesthesia. The cortex and corpus callosum were removed to allow for the placement of electrodes under visual control. Glass microelectrodes configured in 4-barrel array were used for recording and the iontophoresis of diazepam (0.05-0.1M, pH 3.5). Analysis of the diazepam effects were based upon field potential changes in various layers of hippocampal field CA1. Monosynaptically activated field potentials were recorded following microstimulation of the contralateral field CA3.

A paradigm was used which included 7 control stimuli at 0.5Hz, 8 tetanic stimuli at 6Hz, and up to 15 post-tetanic stimuli at 0.5Hz. With the recording electrode in the pyramidal cell layer, and utilizing a stimulus intensity threshold for the appearance of the population spike, the response is dominated by a large positivity. This component appears to be the current flow at high rates of stimulation. While all hippocampal pyramidal cells. The excitatory effect of diazepam on the interneuronal population hereby enhancing pyramidal cell excitability at high rates of stimulation.

This study was supported by NSF GB-55552, Scottish Rite Schizophrenia Foundation, and Hoffman-LaRoche, Inc.

938 PRESUBICULAR, PARASUBICULAR, AND POSTEROMEDIAL TECHENAL PROJECTIONS TO THE RAT HIPPOCAMPAL FORMATION. Konrad Talbot (1) and Glenn J. Giesler, Jr. (2). Department of Psychology, UCLA, Los Angeles, CA 90024(1) and Martin Biological Institute, Galveston, TX 77550(2).

Hippocampal formation (HF: hippocampus + dentate area)afferents have been repeatedly studied in the rat with dianibenzenediol (DAB) horseradish peroxidase (HRP) histochemistry (e.g., Pasquier & Reinoso-Suarez, Br. Res. Bull. 3:375, 1978). Recently, however, Giesler et al. (3) have demonstrated that the deOlmos o-iodanisidine (OD) HRP technique (Exp. Br. Res. 29: 541, 1977) can reveal far more complex neuronal source patterns than do the standard DAB method of Graham and Karnovsky (J. Histochem. Cytchem. 24: 391, 1966). We have thus reexamined HF afferents utilizing OD HRP histochemistry.

Albino rats were injected with 0.04-0.08ul of 30-50 Type VI HRP solutions into (1) medial (N=8) or lateral (N=4) dorsal HF, (2) ventral HF (N=4), or, for control material, neocortex adjacent to the dorsal (N=3) or ventral (N=2) HF. Animals were sacrificed 1-2 days postoperatively and brain tissue reacted for HRP according to the deOlmos (1977, loc. cit.) OD method.

While all differences reported in previous HRP studies were confirmed, several additional input sources were discovered: (1) many cells in the ipsi- and contra-lateral principal external lamina of the pre- and para-subiculum following enzyme injection into the dorsal HF, (2) an appreciable number of neurons in the ipsi- and contra-lateral principal external lamina of the parasubiculum after HRP infusions into the ventral HF, and (3) a small group of neurons in the postero-medial tectal nucleus of Mores (N=3) or great (Lond.J 35: 229, 1961) after enzyme injections into the dorsal and, to a lesser extent, ventral HF, unilateral in the former case and bilateral in the latter.

Two other findings were not predicted by earlier HRP work. First, nerve cells in the principal internal lamina of the entorhinal cortex are labelled by a high enzyme concentration has been delivered to the posterior HF (crus and/or ventral HF). Second, HF neuronal connections linking strictly homolateral HF areas constitute most, if not all, of the nerve cells in the dentate hilus and stratum pyramidale of CA3, as well as an appreciable number in stratum pyramidale of CA2.


This made it possible to study the electrical activity of the brain during free movement behavior. Early spectral analysis studies, after repeated doses of dextro and levo amphetamine, had shown changes in power of the theta, sensorimotor rhythm (SMR) and the 40 Hz rhythms. The loss of power in these signal rhythms seen in the initial treatment with amphetamine showed an increase in power in the second and third doses with a persistence of specific drug related symptoms.

In this paper we are looking at the coherence functions of these rhythms in the same areas of the brain and on the same data used in frequency spectral analysis. Technically, we estimate coherence functions since all of our experimental data are time limited. It is important in relating signals, in recordings of brain electrical activity, to behavioral or drug induced events or programs is very important. A sufficient number of data samples must be averaged to satisfy normal statistical criteria.

During application of amphetamines coherence spectral estimates showed an enhancement of the 30 Hz to 50 Hz frequency range between cortical areas and cortical and subcortical nuclei. There is an increase in coherence at frequencies above 100 Hz. These findings were apparent between the amygdala and nucleus accumbens, amygdala and sigmoid gyrus, and sigmoid gyrus and the same site. Also, there is an increase in power and coherence in the 1 Hz to 5 Hz frequency range. This is associated with increased respiration and heart rate and stereotypy.


We have previously shown that cells of the medial magnocellular reticular formation discharge at high rates during both waking-movement and REM sleep and at low rates during quiet waking and slow wave sleep (Vertes, Brain Res. 128:146, 1977 and J. Neurophysiol. 42:214, 1979). We suggested that these reticular cells may be involved in the generation of hippocampal theta rhythm since they fired maximally during the identical states (general movement and REM sleep) in which theta is selectively present in the hippocampus of the rat.

In the present investigation the involvement of the brainstem in hippocampal theta generation was tested by systematically mapping the entire brainstem with stimulation and determining its effect on hippocampal activity. Under sodium pentobarbital anesthesia rats were fitted bilaterally with bipolar hippocampal electrodes, indifferent and ground leads. Only rats showing distinct high amplitude theta (1 mV or more) in a free moving situation at least one pair of the bipolar hippocampal electrodes were used in the stimulation phase of the experiment. Stimulation was delivered through bipolar electrodes as they were lowered in 0.5 mm steps through tracts (4-6/rat) in the brainstem. Rectangular pulses (0.2 ms, 300 Hz, 50-150 μA) were applied at a rate of 6-5 intervals. The results of stimulating the entire width, depth and length of the brainstem from the bulbo-spinal junction to the caudal midbrain were as follows. (1) Unexpectedly, the most effective sites for eliciting theta were located in medial longitudinal fasciculus (MLF) at the pontine and caudal midbrain level. (2) The only nuclei from which theta could clearly be elicited belonged to the magnocellular reticular chain. The nucleus pontis oralis was most effective in driving theta followed by pontis caudalis and rostral ganglionicreticularis, respectively. (3) Brainstem nuclei such as locus coeruleus, nucleus trapezoid, raphe magnus as well as the lateral reticular areas and cranial nerve nuclei were ineffective in eliciting theta. (4) The only electrolocation that produced desynchronization was located in the median raphe. Desynchronization at this site was accompanied by a dramatic decrease in the amplitude of hippocampal activity. The results suggest that there are separate brainstem systems controlling hippocampal activity—one originating in the median raphe and producing desynchronization and the other in the rostral magnocellular reticular field which fibers primarily course in the MLF producing synchronization.

Supported by grant BNS78-10136, National Science Foundation.


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The postnatal development of the amygdaloid complex was studied in kittens, age newborn to 34 days, using Golgi and EM techniques. The development of the medial nucleus, a phylogenetically older nucleus, and the lateral nucleus, a phylogenetically newer nucleus of the amygdala will be compared in this report.

At birth, all the nuclei of the amygdala which have been described in the adult cat can be identified in the kitten. The neurons in the medial nucleus are stellate shaped and have long fine processes with few spines. The synaptic boutons contained round vesicles and a small population of dark-core vesicles at all ages studied. Many of the boutons were filled with vesicles, others were larger and contained few vesicles in proportion to their size. There was little myelin formation until 23 days of age.

The cells of the lateral nucleus are pyramidal in shape, with thick primary dendritic branches. Long spines with club endings were seen to increase in number up to 14 days of age. At this age, all the medial and lateral nuclei were oriented toward the stria terminalis and a few collateral branches within the nucleus were observed. The myelin in the lateral nucleus is very sparse compared to the medial nucleus. Boutons in the lateral nucleus are similar to those in the medial nucleus and are less numerous.


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In an attempt to develop a better understanding of the functional organization of the hippocampal formation, we employed 14-C-2-deoxyglucose (2DG) autoradiography following electrical stimulation of the various regions along its longitudinal axis in both awake and anesthetized rats.

The experimental paradigm consisted of electrical brain stimulation delivered continuously over 30 sec and 30 sec off for periods of 45 minutes following injection of 2DG. Brains were then removed and processed for radioautography.

The most significant observations were as follows: (1) in unstimulated (control) animals the molecular layer of the hippocampal formation was normally densely labeled; (2) stimulation of the anterior dorsal hippocampus rostral to the level of the subiculum resulted in bilateral activation of all pyramidal cell fields with a marked reduction of label in the hippocampal molecular layer. The dorsomedial septum was only poorly labeled and no evidence of label was seen in the hypothalami; (3) stimulation of the dorsal hippocampal formation at the level of the subiculum and subiculum proper also resulted in bilateral activation of all pyramidal cell fields and a reduction in label over the molecular layer. In contrast, label was clearly noted throughout the extent of the dorsomedial septum and medullary nucleus. There appeared to be no activation of either the ventral hippocampus or any other portion of hypothalamus; (4) stimulation of the pretectal nucleus (at the level of the posterior hippocampus) resulted in activation of only the medullary mammillary and anteroverentral thalamic nuclei. Stimulation of this region produced no diminution in label over the molecular layer nor did it result in activation of either the hippocampal pyramidal cell layer or other regions of hypothalamus; (5) stimulation of the medial septal nucleus at the level of the subiculum proper resulted in activation of the entire extent of the lateral part of the lateral septal nucleus and the medial corticohippocampal pathway; and (6) stimulation of the lateral nucleus produced no diminution in label over the molecular layer.

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Considerable interest exists in sources of sensory afferents to the hippocampal formation and adjacent temporal lobe structures, largely because of this area’s role in memory function.

Anatomical evidence from the monkey indicates that the parahippocampal gyrus may make minor contributions to hippocampal afferents (e.g., Seltzer and Pandya, Exp. Neurol. 50: 146-150, 1976).

Electrophysiological investigation substantiates the view that the responsiveness of neurons in monkey posterior hippocampal gyrus and adjacent areas (McLean et al. J. Neurophysiol. 31: 870-883, 1968) is greater than that of control errors.

AN - ARM MAZE TASK. David Wirtshafter, Karen E. Asin and Ernest W. Kent, Dept. Psychology, University of Illinois at Urbana. We next examined the effects of median raphe lesions in an 8-arm maze task following damage to the hippocampus or a number of closely related structures including the septum and the entorhinal cortex. Since there is evidence that the behavioral effects of median raphe and hippocampal lesions have been reported (Asin et al., this meeting), we investigated the effects of median raphe lesions on the acquisition of a food reinforced 8-arm maze task.

Rats with electrolytic lesions of the median raphe were stereotaxically implanted for chronic recording in the hippocampus, pes hippocampi, and amygdala units indicated that 1) responses were substantially more common to visual than to auditory or somesthetic stimuli, 2) convergence was unusual, 3) responses showed rate increases, rate decreases, or a sequence of both, and 4) the greatest proportion of responsive units was encountered in the posterior hippocampal gyrus. Field potentials evoked by sensory stimuli were of largest amplitude in anterior regions of hippocampal formation, but local responses in anterior hippocampus and amygdala were more apparent than could be predicted from the number of responsive units. Psychically evoked potentials were larger amplitude than those evoked by stimuli of other modalities. Onset latencies ranged from 40 to 120 msec, depending upon the form of the recording that was derived, with shorter latencies in posterior hippocampal gyrus. Preliminary results of tests with patterned stimuli such as reversing checkerboards suggest that responses to such stimuli may not occur in isolation from responses to diffuse photic stimulation.

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948 NEURONAL TRANSMISSION FROM PERFORANT PATHWAY THROUGH DENTATE GYRUS: DUAL BRAINSTEM ACTIVATING SYSTEM. Jonathan Winson, Rockefeller University, New York, NY 10021.

Previous work in this laboratory has shown that the population action potential evoked in granule cells by perforant path (pp) stimulation is greater during the alert state than during the alert state (Winson & Abzug, J. Neurophysiol. 41, 1978). Further, a stimulus applied to the median raphe nucleus (MR) prior to the activation of the pp produces a marked augmentation of the already elevated EAP during SWS but not during the alert state (Winson, Neurosci. Abstr. 3, 1977). In studies reported here, prestimulation (1 or 3 pulses) has been applied to various brainstem locations followed by a test pulse applied to the pp. Extracellular responses were recorded at the granule cell level.

In freely moving rats with prestimulation applied to the MR, the minimum delay time (time from MR to pp stimulus) at which the augmented EAP appeared during SWS was 0.6 sec. It has also been found that a fast field potential (FFP) was elicited consistently at the granule cells, due solely to MR stimulation. The FFP latency was approximately equal (to a phase delay) to a delay dependent on behavior. The FFP was largest during SWS, smaller during alert, and was suppressed during REM sleep.

In rats anesthetized with urethane, midline doroventral-penetrations were made at rostro-caudal levels encompassing the entire pons and medulla to ascertain areas in which prestimulation was any theory of the cellular basis of theta rhythm must account for the fact that the rhythm is phase-reversed between the CA1 and dentate cell layers, yet the phase-locked variations in the amplitude of spontaneous hippocampal theta rhythms are simultaneous in the two cell layers (Fox, Rudell and Ranck, Fed. Proc. 38:1310, 1979). Such a theory must also account for the result that theta cells in CA3 had modes at 100 ± 30° (one was not phase reversed). These results suggest a dual brainstem system activated by midline stimulation which elicits behaviorally dependent responses in the dentate gyrus.

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949 MEDIAN RAPHE LESIONS IMPAIR THE ACQUISITION AND PERFORMANCE OF AN 8-ARM MAZE TASK. David Mirtschbacher, Karen F. Asin and Ernest W. Kent, Dept. Psychology, University of Illinois at Chicago Circle, Chicago, IL 60680.

Electrophysiological investigation substantiates the view that the responsiveness of neurons in monkey posterior hippocampal gyrus and adjacent areas (McLean et al. J. Neurophysiol. 31: 870-883, 1968) in order to 1) compare relative responsiveness of different limbic regions to simple visual, auditory and somesthetic stimuli, 2) determine the relationship between unit and field potential measures of sensory responses and 3) search for receptive field properties during presentation of complex patterned stimuli.

Several authors have demonstrated impaired performance of an 8-arm maze task following damage to the hippocampus or a number of closely related structures including the septum and the entorhinal cortex. Since there is evidence that the behavioral effects of median raphe and hippocampal lesions have been reported (Asin et al., this meeting), we investigated the effects of median raphe lesions on the acquisition of a food reinforced 8-arm maze task.

Rats with electrolytic lesions of the median raphe were stereotaxically implanted for chronic recording in the hippocampus, pes hippocampi, and amygdala. Twelve of 14 seemed to have shown virtually no improvement across 16 days of training. Median raphe lesioned animals showed pronounced response perseveration such that if the rat had entered a particular sequence of arms. Seventy one percent of the errors made by median raphe lesioned animals could be accounted for by such responding, in contrast to only 18 percent of control errors.

We next examined the effects of median raphe lesions in animals who were overtrained on the 8-arm maze task. Despite the near perfect performance of these animals prior to surgery, median raphe lesions produced an immediate disruption of maze performance which was similar in magnitude to that seen in animals lesioned in the entorhinal cortex.

The current results demonstrate that the median raphe, like the hippocampus, contains elements essential to the normal performance of a 8-arm maze task. Despite the near perfect performance of these animals prior to surgery, median raphe lesions produced an immediate disruption of maze performance which was similar in magnitude to that seen in animals lesioned in the entorhinal cortex.

The results of this study demonstrate that the median raphe, like the hippocampus, contains elements essential to the normal performance of a 8-arm maze task. Despite the near perfect performance of these animals prior to surgery, median raphe lesions produced an immediate disruption of maze performance which was similar in magnitude to that seen in animals lesioned in the entorhinal cortex.

To describe the results, the first bin of the histograms was assigned the value of 90° (the positive peak). The mode of the phase histogram was determined for each animal. Although the theta cells in CA1 had their modes at 50 ± 30° (X ± S.D.), four of five theta cells in CA3 had modes at 100 ± 30° (one was not phase reversed). Eight theta cells had modes at 60 ± 30°. Eight other theta cells (not histologically verified) had modes at 60 ± 40°. Firing during REM sleep was recorded in six cells and was the highest during sleep. Slow waves were also recorded in these areas. Twelve of 14 seemed to have phase relations similar to those of theta cells (two had no phase relation). The firing rate of complex theta cells was recorded thus far is too small to make strong conclusions.

Thus both monosynaptic evoked potentials and the firing of theta cells covary with the theta rhythm simultaneously in the dentate gyrus. Furthermore, CA3 theta cells appear to have the same phase relation as those in CA1 and dentate, even though CA3 is more common to visual than to auditory or somesthetic stimuli.

(Supported by NIH Grant NS 13447 and NSF Grant BNS 77-09375 to JBR, and NIH grant NSI 987 to V.E. Amassian.)

Although it has been known for some time that there are cells in the supramammillary region that project to the hippocampal formation (Segal and Landis, 1974; Pasquier and Reinoso-Suarez, 1976), the site of termination of these axons has been difficult to establish. We have examined this issue by placing relatively large injections of tritiated amino acids in the posterior hypothalamus (including the supramammillary region) in a series of rats and cats. In every case in which a significant proportion of the cells in the supramammillary region was labeled, there was a clear indication of axonally transported label in the outer one-half of the stratum granulosum of the dentate gyrus, and over the adjacent 20 µm of the stratum moleculare immediately deep to the zone of termination of the commissural and associational afferents. The projection is to both blades of the dentate gyrus, and extends over the entire septo-temporal extent of the dentate gyrus. However, for reasons that are not yet apparent, the labeling is always appreciably heavier over the suprapyramidal rather than the infrapyramidal blade. In experiments in which the injections were strictly unilateral, labeling in the dentate gyrus was always bilateral; but in every case, the labeling on the side ipsilateral to the injection was significantly heavier than that on the contralateral side.

The supramammillary fibers reach the dentate gyrus by way of the medial forebrain bundle, the septal complex and the dorsal fornix and fimbria. The supramammillary region also projects to the anterior hippocampal rudiment. This projection is similar to that seen in the dentate gyrus, in being both bilateral (but heavier on the ipsilateral side) and limited to the outer part of the cellular layer and the adjoining part of the overlying molecular layer.

References:

SEPTAL CORRELATES OF CONDITIONED INHIBITION AND EXCITATION.
Elna Yadin* and Earl Thomas. Dept. Psych., Bryn Mawr College, Bryn Mawr, PA 19010.

The recording of multiple units was introduced into the study of the role of the septum in Pavlovian conditioned inhibition. This technique enabled the monitoring of septal activity in the freely moving rat throughout a long-term acquisition period of classical conditioning.

In Experiment 1, a Pavlovian aversive discrimination paradigm was used in which one CS was followed by a shock US and another CS was presented unpaired with shock. Another group of animals received a truly random control procedure in which the shock USs were presented randomly with regard to the CSs. Septal activity was recorded during CS+ and CS-, during a preCS period and during a post-shock period.

The results may be summarized as follows: (1) septal unit activity was found to increase during presentations of CS- only in the conditioning group. (2) Presentations of CS+ caused a marked suppression of septal unit activity in the conditioning animals. (3) The baseline unit activity during the preCS period remained unchanged throughout the experiment in the conditioning animals while decreasing in the truly random controls. (4) Termination of the shock US was marked by a large burst of firing in the conditioning group but no such rebound was found in the controls.

The results are interpreted in terms of a fear-relief hypothesis which suggests that the septal area is importantly involved in the mediation of relief from fear. The differential patterns of firing between the experimental and control groups suggest the importance of the predictability of shock to the expression of relief.

In Experiment 2, a Pavlovian appetitive discrimination paradigm was used in which the CS+ was followed by a food US. The results were virtually the opposite from those seen in the aversive case: (1) Septal activity to CS- was suppressed. (2) Unit responding to CS+ was markedly enhanced.

The findings from the appetitive experiment were interpreted as indicating that increased septal activity during CS- seems to be quite specific to the nonoccurrence of a noxious stimulus and does not represent the mechanism underlying all types of conditioned inhibition.
MEMBRANE
BIOPHYSICS
CALCIUM CHANNELS PERMIT THE PASSAGE OF Mn++ IONS: A POSSIBLE MEMBRANE CURRENT IN INSECT MUSCLE FIBRES. Frances M. Ashcroft*

The myoepithelial cells that make up the proventriculal of the marine polychaete worm Syllis spongiphila undergo calcium spikes which are associated with contactations. The spikes elicited by direct intracellular stimulation are (a) reversibly abolished in Ca-free artificial sea water (ASW), (b) not abolished in low-sodium ASW or Mg++-substitution ASW, (c) abolished reversibly by Ca++ ions, 6-100 µM and verapamil (1 µM) and essentially irreversibly by La+++ ions (10 - 100 µM), and (d) supported in Ca-containing ASW containing either 1 mM Ba++ or 10 mM Sr++ ions. Mn++ ions appear to pass through the calcium channels. (a) Regenerative responses not associated with contractions can be elicited in Ca-free ASW containing 5 - 50 mM Mn++. (b) Ca-free ASW containing Mn++ ions, spikes are abolished by Co++ and La+++ ions, 6-100 and verapamil at concentrations similar to those effective in abolishing Ca-spikes. (c) In Ca-free ASW containing varying [Mn]o's, amplitudes of overshoots increase about 15 µV for a 10-fold change in [Mn]o (these values approach that predicted by the Nernst equation for a membrane permeable to a divalent cation); in ASW containing 10 mM Ca++ and varying [Mn]o's, the amplitudes of overshoots increase about 15 µV for a 10-fold change in [Mn]o (these data suggest a compe­

The divalent first transition series metals, Mn++ exhibits the lowest energy of hydration. It seems probable that Mn++ ions can relatively easily shed their waters of hydration and pass through the Ca-channel. On the basis of this hypothesis, divalent cations of the first transition series with higher energies of hydration should block the Ca-channel. The effectve­

of blocking should increase as the energy of hydration increases; the prediction of effectiveness would be Fe++ < Co++ < Ni++.

The relative effectiveness of Fe++, Co++ and Ni++ ions was tested by applying a series of concentrations of each ion to the prepa­

ration in Ca-containing solutions and determining the concen­

tration required to abolish Ca-spikes elicited by direct stimula­

tion. (During the application of Fe++ ions the preparation was

bathed in nitrogen-bubbled, low-oxygen solutions). Ni++ (1 - 10 µM) consistently abolished the Ca-spike at lower concentrations than Co++; Co++ (5 - 20 µM) abolished the Ca-spike at similar or lower concentrations than Fe++ (10 - 20 µM). These data support the idea that the capability of an ion to block the calcium channel increases as the energy of hydration increases.

Supported by USPHS Grant R 5 NS12196.

MEMBRANE CURRENTS IN INSECT MUSCLE FIBRES. Frances M. Ashcroft* and R.R. Stanfield* Dept. of Physiology, University of Leicester, Leicester, U.K. (SPON. G.F. Gwilliam). Membrane currents in the ventral longitudinal muscle fibres of the stick insect, Carausius morosus, were investigated with a 3-electrode voltage clamp. Experiments were carried out at 2-5ºC, in hypertonic saline to block contraction. The membrane current consisted of an early inward current, an early outward current and a delayed outward current. The presence of an early outward current is probably responsible for the graded (as opposed to all-or-none) action potentials which are charact­

eristic of insect muscle. The inward current was studied in Ringer containing 120mM TEACI to reduce outward currents. In nor­

mal Ringer (20mM CaCl2) the maximum inward current was -77.6 ±

5.9 µA/cm² (n=10) at a membrane potential of 0mV. Peak inward currents were increased in 50mM Ca2+-Ringer (Mg2+­substitution) and reduced in 5mM Ca++-Ringer, and there was a 31.0W shift in the potential at which the membrane current reversed in sign for a 10-fold change in external calcium. At high [Ca++], the peak inward current showed saturation. Inward currents were blocked by 1mM La3+ and halved by 10mM Ni2+. These results indicate that the inward current is carried by calcium ions. The inward current (Ica) shows voltage and time-dependent inacti­

vation. Steady-state inactivation was described by the equation:

Ica = Ica0 [1-exp (-Vh/VT)] where Ica0 = 21.0±1.8W (n=5) and VT=85. This time constant of inactivation depended on the experimental protocol. Voltage dependence of a time constant of 55msec at -10W when determined with 2-pulse experiments; however the decay of Ica during a single voltage step was best fitted by the sum of two exponentials. The time constant of the Ca channel are currently under study. As insect muscle is readily voltage-clamped it provides a favourable preparation for the study of muscle calcium channels. Supported by the M.R.C.

Recent interest in whole-body exposure to time varying magnetic fields has been stimulated by the proposal and development of novel biophysical techniques using nuclear magnetic resonance. Some fields require rapidly ramped magnetic fields. Theoretical insight can be gained into the circumstances under which such exposure will stimulate excitability of neural tissue. The problem of field stimulation is taken to arise from the electric fields created by exposure to time varying magnetic fields. The assumptions of two present treatments are standard for a spherical, laminar flow of nervous tissue. It is presumed to have a threshold value of transmembrane depolarization, ΔV_c. The cell is taken to be in a medium of uniform electric and magnetic properties and all calculations are made for steady excitation, i.e., the rheobase limit. Two cases are considered: the direct excitation of a cell body and excitation of an extended process—an axon or muscle fiber. Consider a spherical cell body of radius r in a uniform field E, the potential difference between inside and outside, ΔV, in mV that is 20-100 times greater than the slope at potentials near 0 mV. Thus, the amplitude of the calcium current in Ca-free external solution, Dr. Byerly, and a CNPq fellowship (Brazil) to Dr. Masuda. There is a striking correlation between the amplitude of the calcium current and the Cs-mediated outward current. Any treatment resulting in a change in membrane conductance to potassium ions (Brehm and Eckert, 1978; Eckert and Brehm, Ann. Rev. Biophys. Biomech., 1979). Following EGTA injection, the peak P current resulting from presentation of P. These treatments were compared to experiment.

958 ELEVATION OF INTERNAL FREE Ca++ IS REQUIRED FOR INACTIVATION OF Ca CONDUCTANCE IN PARAMECIUM. P. Brehm* and R. Eckert (Sponsor: D. J. Junge) Dept. Biology, University of Los Angeles, CA.

Inactivation of the Ca conductance in Paramecium does not exhibit the voltage dependency characteristic of Na channels. Instead, the inactivation requires the presence of Ca (Brehm and Eckert, Science 202:1203-1206, 1978). To further investigate the role of Ca, we injected the Ca chelator EGTA to limit the elevation of free Ca within the cell during Ca entry.

Specimens were clamped with holding voltage equal to

\[ \text{In nM CaCl}_2 + 4 \text{ mM KCl} + 1 \text{ mM HEPES at pH 7.2.} \]

EGTA was used to chelate Ca from the compound eye of a paramecium filled with 100 mM K-EGTA while under steady voltage clamp. Inactivation of Ca current was measured before and after EGTA chelation, when the cell was recorded for 20 msec depolarizations, P1 and P2, were delivered with a 40 msec interval. P2 was fixed at 30 mV, a level at which little late outward current was observed. P1 was presented over a wide range of potentials and the late outward current remaining after the 40 msec interval was determined from the peak P peak current resulting from presentation of P1. From the P2/P1 ratio, we found the midrange of Pi potentials (Fig. B). Changes in P2/P1 were compared to experiment.

959 Cesium Carries Large Outward Currents in Internally-Dialyzed Snail Neurons. Lou Byerly, Daisun Tagawa, Masako O. Masuda*, and Mitsunobu Yoshit*.

We have applied a modified version of the internal-dialysis, voltage-clamp technique of Brown and Brown (J. Gen. Physiol. 21:489, 1978) to ganglion nerve cell bodies of the freshwater snail, Limnea stagnalis. The ganglia are treated with trypsin to facilitate isolation of the cell bodies and to increase the periphery and is given by the formula

\[ E = \frac{10^{-8}BR}{2} \]

Equating the electrostatic equilibrium between the inside and outside, ∆V, is ∆V = (3aE/2)cosθ' where ∂ is the angle that an internal radius vector makes with the field direction. The incorrect approximation that the cell is uniformly excitable, the critical field for excitation, E_c, is \( E_c = \frac{2\Delta V_c}{\lambda} \) [1/(1-exp(-L/λ))]. The factor of the second parenthesis approaches unity as L/λ>>1. To connect this criterion to cells, assume a defined electronic coupling distance, λ, for small size of most cell bodies this excitation limit is much higher than that for fibrous structures. For an extended process we assume a defined electrical coupling distance, λ, for small signals. Further, it is taken that the electric field, E, makes an angle θ with the fiber axis and sensibly exists over a length L of the long fiber. A remarkable early paper by W.A.H. Rushton (C. R. Physiol. 63, 1927) has pointed out that the transmembrane potential difference between inside and outside, ΔV, in mV that is 20-100 times greater than the slope at potentials near 0 mV. Thus, the amplitude of the calcium current in Ca-free external solution, Dr. Byerly, and a CNPq fellowship (Brazil) to Dr. Masuda. There is a striking correlation between the amplitude of the calcium current and the Cs-mediated outward current. Any treatment resulting in a change in membrane conductance to potassium ions (Brehm and Eckert, 1978; Eckert and Brehm, Ann. Rev. Biophys. Biomech., 1979). Following EGTA injection, the peak P current resulting from presentation of P. These treatments were compared to experiment.

960 A COMPARISON OF OUABAIN-SENSITIVITY AND HYPOXIA ON THE INTRACELLULAR POTASSIUM ION ACTIVITIES IN IDENTIFIABLE NEURONS OF Aplysia californica. Philip E. Cover*, Dept. of Neur. and Neurosciences Program, Univ. of AT. Medical Center, Birmingham, Alabama 35294.

In different types of neuronal activity, either regenerative action potentials or decremental generator signals, hypoxia often causes membrane depolarization and concomitant changes in the action potential frequency or the amplitude of the potential. Three independent explanations or possibly a combination thereof may account for the observed phenomena: (i) a decrease in the electrogenic pump resulting in (ii) a change in the equilibrium potentials of the principal ions contributing to membrane potentials or (iii) a membrane conductance change. To test these hypotheses, identifiable neurons from the abdou nal ganglion were impaled with double-barrel, potassium ion-selective microelectrodes capable of measuring intracellular potassium ion activities and membrane potential. Neurons were maintained in a constantly-suffused chamber supplied by normal bathing solution held at 18°C. After nitrogen bubbling, gaseous equilibration of the normal saline was detected by pD microelectrodes placed in the recording chamber. While the relative potassium ion activities on either side of the neuron's membrane were determined with the ion-selective electrodes, external sodium, calcium, and potassium ion concentrations were altered. Upon calculating the potassium equilibrium potential at the point of the after-potential or, relative to the membrane conductance (see figure), the effects of hypoxia were compared to that of 4x10^-4 M NaCl exposure (open triangles). Control data were obtained by subjecting the neuron to a 10-sec hypoxia (open symbols), control experiments in which the external potassium ion concentration was lowered by a hyperpolarizing current pulse resulted in spiking indicating that a change in membrane conductance to potassium had occurred. The close agreement of its slope and that of the expected result (solid squares fit slope of dashed line) indicates that the potassium had occurred. Removal of the inactivation factor by a hyperpolarizing current pulse resulted in a change in conductance and not the equilibrium potentials had occurred.
A qualitative hypothesis, based on molecular orbital theory, is proposed to account for the alkalai metal ion selectivities exhibited by the macrocyclic antibiotics valinomycin and emimatin B. Even though the complexes formed between the metal ions and the macromolecules are of low molecular symmetry, the bonding parts of the complex have relatively high local symmetries. Spectroscopic studies have shown that both valinomycin and emimatin B, the six oxygen atoms which bind the central cation, form a trigonal antiprism (Shemakin, M. et al., J. Membrane Biol. 1:402 (1969)). Therefore, the bonding parts of the complex are assumed to belong to the D₃h point group. Binding is explained by the sharing of electrons from the oxygen atoms of the macromolecule and the vacant valence orbitals of the cation. Group theoretical methods are used to determine the allowed orbital interactions. The observed specificity is explained in terms of the bonds that are symmetry-allowed and qualitative considerations of orbital overlap and steric effects. On this basis, the observed selectivity sequences of the macrocyclic antibiotics can be accounted for. Experimental ways of verifying the assumptions proposed to explain the specificities are also suggested.

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Contribution No.88 of the Laboratory of Neurobiology.

Figure: Effect of 10⁻⁵M noradrenaline (arrows) on the duration (A) and inward Ca²⁺ current (B) in TTX. Cal: A=20mV,5ma; B=50V/50ma,2ms.

According to conventional kinetics, the rate coefficient of a primary process is constant during the progress of the process. When this assumption is found to be incorrect, ad hoc adjustment is made. Thus, in Hodgkin-Huxley type equations, rate constants, implicit in initial assumptions, generate m,n,h processes, but their changing relation to Vₘ is subsequently found empirically.

A more realistic initial assumption is that the rate coefficient of a primary process changes systematically during the course of the event, because conditions change as the system adjusts to a new equilibrium or steady-state. Rate constants then specify the rate of change of the rate coefficients of the primary processes. This assumption, yielding simple equations of great power, has been used to model the conductance data of Hodgkin and Huxley, as well as oxyhemoglobin dissociation curves (Raston, Biophys. J. 22, 1978; J. Theoret. Biol. 76, 1979). The assumption that the rate coefficient changes exponentially with the independent variable is particularly well suited to such systems, involving asymmetric sigmoid relations between dependent and independent variables. Current-voltage-time records of voltage clamp data can now be modelled directly (rather than via conductance) from the following assumptions of the new (e xo-exponential) kinetics: following a voltage step, current increases exponentially, but the rate coefficient of that growth decreases exponentially with time and increases exponentially with voltage. From this assumption, prediction of the V clamp results may be made by use of a single equation (with appropriate constants) derived to describe either specific ion current Iₖ or I₉₆. (Aided by Psychobiology Center and Computer Center, Florida State University)

Impulse trains (fig. 1) generated by the space clamped squid giant axon in response to, and phase locked to the current I(t) = I_{0}(sin2πt/5) were modulated by a second, small amplitude sinusoidal current. The resulting impulse density was estimated for a complete period of the modulation sine wave from an impulse train with a duration of many modulation cycles (fig 2). For sufficiently small modulation amplitudes this impulse density function is itself a sinusoid with the same modulation period. Oftentimes one observes that a corner frequency and a gain resonance determine the magnitude of membrane conductance. The phase curve is a function of the conductance time course. Additional structure in the Bode plots may suggest the occurrence of "summing" phenomena (variables which are not reset by an impulse), and specify the relaxation times of those variables. The Bode plots of the squid axon membrane (fig. 3, for example) and of several models including the Hodgkin-Huxley model (fig. 4, for example) are qualitatively different. The necessary stimulus current and the measured conductance level are considerably higher for the axon. More importantly, the relaxation times of "recovery variables" (K-conductance and/or Na-inactivation) appear to be considerably longer for the axon when stimulated with sinusoidal currents than those of models derived from voltage-clamp data and "stimulated" with the same currents.

SENSITIVITY OF A Na+ PUMP TO MEMBRANE POTENTIAL. E. F. Holloman, S. D. King, Iola of Neurophysiology, University of Minnesota, Minneapolis, MN 55455.

In the crayfish stretch receptor neuron, a train of antidromically evoked action potentials is followed by a post tetanic hyperpolarization that has been shown to be due to the action of an electroneutral Na+ pump. The same phenomena also underlies a post tetanic depression of firing in an active neuron (A below) and also the adaptation of firing to a constant current (C below). It has been proposed that the small sodium influx associated with each action potential is sufficient to trigger the electroneutral pump. Alternatively, the following evidence suggests that the electrogenic pumping is activated by the changing electric field associated with the action potential.

The application of a hyperpolarizing pulse shortly after an action potential will not affect the sodium influx but will alter the electric field across the membrane. As shown below, a hyperpolarizing pulse (100 mV in amplitude, 5 ms in duration; applied intracellularly to the neuron soma 10 ms after each antidromic action potential) abolishes the post tetanic action potential normally observed after a 1 s antidromic train (50/s) (B). The adaptation to a step change in current can also be abolished when the hyperpolarizing pulse is applied after each action potential (D).

Supported by grants from NSF. BMS 77-22532 and PCM 78-25168.


The time-course of anomalous rectification potassium current of either twitch fibers was analyzed using Hill and Campbell type voltage clamp, which gives a time resolution of 1-5 msec. When muscle fibers are hyperpolarized an "instantaneous" inward current is observed, which is followed by a time dependent increase of the current. Both "instantaneous" and steady-state I-V relations have the property of an inward rectifier. The time-dependent current can be described by first-order kinetics. The "instantaneous" I-V relation may reflect the inward rectification of a single channel. Alternatively, the "instantaneous" I-V relation might reflect a change in the number of open channels which is complete within a few msec, and thus cannot be resolved with the present technique. When Ca++ ions are present in the external solution a decrease, or blockage of inward rectification is observed. As the membrane is hyperpolarized to a more negative potential a reduction of the current is observed. This voltage-dependent blockage has the property of an inward rectifier. The negative slope region in the steady-state I-V relation when the membrane potential is stepped between two levels, the ratio of blocked to unblocked channels is changed in a time-dependent process.

Inward rectification channels of starfish egg cells (Hagiwara et al., J. Gen. Physiol. 67:621, 1976) might indicate that Cs+ blocking and channel gating are separate processes. However, the time course of the conductance increase is more than ten times faster in muscle fibers, but the kinetics of blockage by Cs+ is on the same scale of magnitude. This suggests that Cs+ blocking and channel gating are separate mechanisms.

Supported by NSF grant NS09012 to Dr. Hagiwara and NIH Department Training Grant 5 T01 GM 00448.

VOLTAGE-CLAMP ANALYSIS OF CA3 NEURONS IN HIPPOCAMPAL SLICES. Daniel Johnston and John Hablitz. Dept of Neurophysiology, UCLA Medical School, Los Angeles, CA 90024.

The time-course of anomalous rectification potassium current was analyzed in hippocampal CA3 neurons in acute slices. The CA3 pyramidal neurons in the hippocampus fire in spontaneous bursts of action potentials (APs) at fairly regular intervals. However, the time course of the conductance increase is on the same scale of magnitude. This suggests that Cs+ blocking and channel gating are separate mechanisms. However, the time course of the conductance increase is more than ten times faster in muscle fibers, but the kinetics of blockage by Cs+ is on the same scale of magnitude. This suggests that Cs+ blocking and channel gating are separate mechanisms.

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Astroglia are known to readily swell in vivo when the central nervous system is subjected to a variety of insults. Their perivascular location also well suited the role of a protective extracellular fluid and pH control. These effects are clearly based on the ion transport characteristics of these cells and we have previously discussed the role of Ca in astroglial swelling. Since ion transport in glia is difficult to define precisely in intact tissue, we are using a primary culture started from the cerebro hemispheres of 1-3 day old rats, which we have isolated in vitro model of astroglial cells. We have found that 70-90% of the cells in these cultures stain positively for glial fibrillar acidic protein (GFAP) by immunofluorescence.

The rate of the steady state unidirectional efflux or influx of Cl⁻, measured with 36Cl⁻, was reduced by up to 50% by 0.1 to 1.0 mM of the unlabelled SITS or 4,4'-diisothiocyanato-2,2'-stilbene disulfonic acid (DIDS) for Cl⁻ transport. Tetramethrin, which was substituted for K⁺, was also effective in inhibiting Cl⁻ transport. A concentration of 10 µM tetramethrin reduced the efflux of Cl⁻ by 50%. These data suggest that a significant amount of Cl⁻ transport in these cells occurs by a mediated process, possibly involving an exchange carrier similar to that found in red blood cells. The intracellular chloride concentration, based on an estimated internal volume of 4,81±0.2 µl/mg protein from 8% distribution studies, and a Cl⁻ content of 0.145±0.002 µM/mg protein from steady state 36Cl⁻ levels, was calculated to be 380mM. This was 3 times greater than would be in an equilibrium with a measured average membrane potential of ~70 mV. Other indications that [Cl⁻]i was not at a high level was that steady state [Cl⁻]e was the same for [Cl⁻]o reduced to 0.05 mM or with [Cl⁻]o increased to maintain a constant external K⁺ to Cl⁻ ratio. Furthermore, pretreatment of the cells with ouabain, which resulted in a reversal of the normal high intracellular ([Glu⁺]i) to extracellular ([Glu⁺]o) ATPase, had no effect on Cl⁻ uptake or final steady state levels as measured with 36Cl⁻. At the present it is unclear whether the high [Cl⁻]i is due to operation of a Na⁺/Cl⁻ pump or to increased uptake of a Cl⁻-sensitive carrier in exchange for another anion, such as HCO₃. In brain slices, however, the occurrence of HO₃⁻-depend­ent and SITS-sensitive swelling involving increased NaCl uptake and swelling of astroglial cells, suggests exchange of intracellu­lar HO₃⁻ for Cl⁻. Since addition of NaCl to cells incubated in NaCl-free medium led an increased rate of acidification of the medium, increased uptake of Na⁺ could be due to NaCl→Na⁺ exchange. Supported by NIH grant 13042.


A variety of natural and synthetic toxins have proved useful in studying ionic conductance mechanisms in nerve mem­branes. They synthetic pyrrothid insecticides are a relative­ly new group of toxins with potent and unique actions on neuronal membranes. Since the superexcitability accompanying interlaminar slow axonal activity is observed with the same volt­age clamp technique, we have studied the effect of one of these, tetramethrin, on crayfish giant axons. Median giant axons were isolated from the median nerve of a Spiny告诉我们 crayfish and perfused with a Ringer's solution of 220 mM K⁺, 15 mM Na⁺, 170 mM glutamate, 50 mM F₄, 15 mM Cl⁻, and 96 mM sucrose with a pH of 7.3. The holding membrane potential was ~100 mV for all experiments. Tetramethrin applied at a concentration of as high as 300 µM internally or extracellularly, had no effect on the time course or the steady-state amplitude of the sodium current measured in the presence of 300 µM tetrodotoxin. However, the (*) optical isomers of tetramethrin had a specific effect on the sodium current as measured with an internal perfusate in which Ca was substituted for K⁺. A concentration of 10 µM applied to either side of the axonal membrane caused the inward sodium currents to become biphasic, a normal transient sodium current being followed by a secondary inward current of much slower time course. This slow inward current was observed without the initial sodium transient at membrane potentials below ~70 mV. Thus the voltage dependence of the slow current is shifted in the direction of hyperpolarization with respect to the initial transient sodium current. The time course of the sodium tail current, however, followed the order kinetics in the control axon, became the second order in the tetramethrin-treated axon. The first time constant (~1 msec) coincided with the control tail current and the second time constant was on the order of hundreds of milliseconds. Increasing the concentration of tetramethrin had no effect on the tail time course, but increased tail currents of the same amplitude. Experiments in which depolarizing pulses of varying duration were applied showed that the development of the slow phase of the tail current was not influenced by the development of the slow inward current. These observations suggest that a population of sodium channels is modified by tetramethrin to give rise to drastically slowed activation and inactivation.

The slow channels* activated at a time course of 50-500 msec and inactivate with a time course of several seconds. Supported by NIH grant NS14143.

Slow membrane potential shifts in cortical neurons of the conscious cat in response to meaningful stimuli. Gregory L. King* and James E. Skirimer, Neurophysiol. Sect., Neurology Dept., Baylor College of Medicine, Houston, Texas 77030.

When a conscious animal or human is presented with a tone that forms part of a classical conditioning, a novel object, or a strongly stimulating an extracellular slow potential (SP) is evoked in the frontal and parietal association cortices. In different situations in which the same stimulus is given, meaning that the non-specific sensory input are therefore evoked by the context of the stimulus, not its physical attributes. Current evidence suggests that these cere­bral slow event-related SP's result from an intracellular mechanism that is thought to produce SP's in the sympathetic ganglion neurons. In the isolated ganglion the SP is accompanied by slow postsynaptic potentials and is correlated with shifts in the intracellular levels of cyclic AMP (cyclic 3',5'-adenosine mono­phosphate). Our laboratory has shown that the amplitude of an event-related SP in the rat parietal cortex is negatively corre­lated with the local tissue level of cyclic AMP (Kimmer et al., J. Neurochem., 1978). Our most recent observations show that slow 20-30 sec membrane depolarizations occur in some neurons in the frontal cortex of the conscious cat during a surface-negative event-related SP that is recorded from the same local area (Figure 1). These large depolarizations are associated with a reduction in the spontaneous action potential discharges, and, in some cells, are accompanied by a conductance increase. Other cells do not manifest detectable changes in the current-voltage relationship during the evoked slow membrane potential shift. By employing a "floating" microperfusion method of recording, we have been able to perform several replications of the observations in the same cell.

Figure 1

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Slow long-term aftereffects of subthreshold electrical stimulation of peripheral nerve axons. Kenneth McLeod and Stephen H. Raymond. Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

In cat dorsal horn, Merrill, et al., reported aftereffects that linger for 20 msec or more following single subthreshold electro­cal pulses (J. Physiol. 284:127-145, 1979). Gasser (Am.J.Physiol. 121:193-202, 1938) showed indirect evidence for such long-term aftereffects in his study of "recruitment" of nerve fibers in whole nerve given near threshold tetanic stimulation. We find that when an excited frog sciatic nerve is bathed in Boyle-Conway Ringers at constant pH 7.0-7.7 and constant temperature, stimulation of the nerve with current pulses that are near or below threshold results in brief transient increases in excitability that decay within 2 msec. The last stimulus in a short burst of such subthreshold stimulation is followed by an enduring rapid decrease of excitability that is characterized by a single pulse or the last stimula­tion of a subthreshold stimulus. Under these conditions there are no detectable long-term aftereffects of subthreshold stimu­lation as assayed either by threshold hunting or by raising the amplitude of subthreshold stimuli gradually.

The pH of the Ringers solution was set by adjusting the mixture of 5% CO₂ in air, 30% CO₂ in O₂, and O₂ bubbled through reser­voirs of Ringers. After several hours at pH 9.0, rapid restora­tion to pH 7.5 produced prolonged rises in threshold lasting for more than an hour. During such depressed excitability, individual fibers given repeated stimulation showed a superexcitability as high as 20% below resting level that was detected for more than 50 msec following the last pulse in a train. This superexcitability appears to account for recruitment.

1) It appears for the first pulse after threshold results.
2) It lasts about as long as the maximum period between stimuli in trains that produce some recruitment in gross sciatic nerve. (The threshold amplitude of single pulses is often not decreased as the rate of recruitment) as the period between stimuli gets longer.

We are investigating relations of pH-induced depression to depression induced by furosemide, an amphoteric protein (Stieg et al. this volume).

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973 **COMPARTIMENTAL ANALYSIS OF ELECTRICAL CONSTANTS IN CULTURED MOUSE DORSAL ROOT GANGLION NEURONS. Jane C. Norris*, Thomas H. Brown and Donald B. Pappas**. Dept. of Neurology and Biophysical Sciences, Stanford University, Stanford, California 94305

Cultured mouse dorsal root ganglion (DRG) neurons offer both experimental and physiological advantages. They are utilized for evaluating the electrical constants in a mammalian neuron. Their simple geometry makes it possible to represent the cell by an isopotential, soma-compartmentated cylinder of a cell body (with a conductance \( G_s \)) which is attached to a single dendritic cylinder (represented by a resistive-capacitive network in parallel with the soma compartment) having an electron (length \( L \)). We have developed and applied to these cells a convenient method for obtaining specific membrane properties and electrotonic structure.

The DRG cell soma was penetrated with two independent microelectrodes, one for delivering current steps and the other for recording voltage responses. Current-voltage curves were obtained and subsequent analysis was restricted to the voltage range within which the cell membrane gave an ohmic or linear response to hyperpolarizing current steps. The slope of the hyperpolarizing charging curve was used for the function

\[
\frac{dV}{dt}(t) = -\frac{C_m}{R_m} e^{-t/\tau_1}
\]

where \( \tau_1 \) is the time from the onset of the current step. The dendritic to somatic input conductance ratio \( \rho \) was obtained from the relation

\[
\rho = \frac{G_{ds} - 1}{G_m}
\]

where \( G_s \) is the input conductance of the whole cell, \( \tau_m \) is the membrane time constant, and \( \lambda \) is the amplitude of the current step. The remaining parameters could then be obtained as follows:

\[
G_s = \frac{G_d}{(1+\tau /\tau_m)} \quad G_m = \frac{G_s \lambda}{(1+\tau /\tau_m)} \quad L = \frac{(G_d + G_s \lambda)}{(\lambda \tau_m)}
\]

where \( \tau \) is an "equalizing" time constant. The cell soma area \( A_s \) was determined and, from \( A_s \) and the values of \( G_s \) and \( G_m \), we calculated the input membrane resistivity \( R_m \) and specific membrane capacitance \( C_m \).

All of the electrical constants were within the usual range for a cerebellar neuron, except for \( \rho \), which was between 0 and 1. The assumptions underlying this analysis were assessed with computer simulations, using the compartmental analysis of Perkel and Nicolai (J. Physiol. 278, 1974), and were found to be reasonable for cells with the general shape of a DRG neuron.

The applicability of these analytical methods to some neurons with very different cellular geometries is currently under investigation. (This research was supported by NS 06161, NS 12151 and NS 09744).


The action potential recorded from HIE-115 neoblastoma cells exhibits a fast depolarizing phase followed by a transient repolarization, and finally a depolarizing after-potential that decays to the resting membrane level in 0.1 to 10 sec. Through voltage-clamp experiments, Moolenaar and Spector (J. Physiol. 278, 265, 1978) found voltage-dependent Na, K and Ca channels in these cells. We have now studied some of these channels at a normal pressure of 760 mm Hg. The effect of pressure was to increase the membrane conductance, except for Na, which was decreased. The action potential was prolonged by increased pressure: pressure produced a reduction of excitability and a delayed after-potential of the action potential. This increase in excitability was associated with an increase in potassium conductance in the squid axon and an increase in the ultra-slow outward current, thought to be carried by potassium in the giant cells of Aplysia. The rate of adaptation of the Aplysia neurons was increased by pressure in a manner similar to that caused by the anesthetic. When pressure and the anesthetic were applied simultaneously both effects were noted, and no indication of pressure reversal was apparent. In earlier experiments we have assumed functionally distinct, so that for a "critical volume hypothesis" may have to be redined to account for pressure reversal observations in whole animals.
A modification of the technique of Neher and Lux (Pflugers Arch. 311: 272, 1969) was used to measure current through small (5-µm diameter) membrane patches in tonic abdominal stretch receptor neurons of the crayfish Palaemonetes paludosus. Two intracellular electrodes were used to voltage clamp the cell. The extracellular patch electrode was positioned close to the intracellular voltage electrode; the membrane was not well clamped at distances greater than 50 µ.

Three major components of the active spike currents in the dendrites, soma, and proximal axon were found: a non-inactivating outward K+ current and two transient inward NMDA currents. Both of the inward components are present in the soma (A), while the axon (B) shows only the fast component and the dendrites (C) show only the slow component. The outward current was present at all locations studied. Both inward components were blocked by 2 mM-TEA and showed similar dose-response characteristics. Both were abolished in Na+-free (choline substituted) saline and were unaffected by Li+ substitution for Na+, by low Ca++, or by addition of Ba++ or Co++. The two inward components observed in the soma could be separated by applying a 4 msec duration depolarizing step to inactivate one component completely. By this time the fast component had recovered while the slow component was still inactivated.

The slower inward current in the soma and dendrites causes a broadening of the dendritic spike and often produces a notched waveform of the soma spike. This current is probably involved in double-spiking seen at elevated temperature (Calvin and Hartline, J. Neurophysiol. 40:106, 1977). A similar mechanism could be present in other neurons which show notched soma spikes and double-spiking (Calvin and Sypert, J. Neurophysiol. 39:420, 1976).

Capacitance measurements give values of 2-5 µF/cm2 (compared to 1 µF/cm2 found in most lipid bilayer membranes), suggesting a substantial infolding of the cell surface.

VOLTAGE CLAMP STUDIES OF A-CURRENT VARIABILITY IN NEURONES OF ARCHIDORIS MONTEREYNSIS. Elba E. Serrano* and Peter A. Getting. (SPONS: P. Lennard). Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Voltage clamp studies of molluscan neurones demonstrate the existence of severalionic currents with voltage and time dependent conductance changes. Presumably the characteristic firing patterns of cells can be explained by the integration of these ionic currents in space and time across the membrane. Differences in the kinds of currents, their relative contribution to membrane excitability and the parameters of the time course of the current through the membrane can account for the intercell variability of the A-current system under voltage clamp. Furthermore, the mutant and the wild type have the same maximal GCa.

The maximal GCa is not affected by the growth temperature in both wild type and the teaB mutant, but is about 10 mV more positive than that of wild type. The inactivation curve (max GCa-Vh relation) of the mutant is about 8 mV more positive than that of wild type. However, the mutant and the wild type have the same maximal GCa of 40 nmho/cell. The K conductance responsible for the anomalous rectification also appears more stabilized in the mutant.

Wild-type paramecia grown at a high temperature (34°C), but tested at room temperature (22°C), differ from those that never experienced the high temperature. The GCa-V relation shifted to the left by 8 mV in the teaB mutant, however, shows little change after incubations at 34°C. The maximal GCa is not affected by the growth temperature in both the wild type and the mutant. It is known that chilled protozoa can detect temperature and redirect their phospholipid fluidity. The teaB mutation may damage this mechanism and cause a loss in thermoresponsiveness (corresponds with the behavioral observation by T. Hennessy). This would lead to perturbation in membrane lipid compositions, surface-charge pattern and membrane stability.

The experiments were done under a voltage clamp. The Ca currents were given by the subtraction method using non-leaky potassium, ND4AS, 5mM NaCl, a defective site on a number of functional Ca channels (Satow & Kung, submitted to J. Exp. Biol.) and +115 mV for ECa was used for the calculation of GCa as a chord conductance. The external Ca concentration was 0.91 mM and the external K concentration was 4 mM through the experiments. This work was supported by NSF grant BNS77-20440 to C. Kung.

Isolated neurons from the cerebral, pleural and pedal ganglia of Archidoris montereynsis were voltage clamped at 10°C. The population of cell types is comprised of five neurons. Cells were chosen on the basis of constancy of location from prep to prep, ease of removal from the ganglion, reproducible firing patterns. Between cell types, however, there is a variation in the f-I responses under current clamp. Variability has been examined in the following parameters:

- a) magnitude of currents b) steady state activation and inactivation c) peak current:activation and inactivation d) time constant for decay e) time constant for activation f) time constant for removal of inactivation. In this group of cells, the variation in the activation and inactivation characteristics is within cell type, and may be a result of the same kind of variation observed in variability between cells. The most striking variability between cells seems to be in the kinetics of their response to changes in potential. In particular, the time constants for slow current were measured from a cellular and variability between cells. Cells segregate into two populations: in one, the t<sub>g</sub> is of the order of 60-90 ms, in the other, it is between 240 and 330 ms.

A MUTATION AFFECTING MEMBRANE STABILITY IN PARAMECIUM TETRAURELIA. Youko Satow. Lab. Molecular Biology, Univ. Wisconsin, Madison, WI 53706.

It is well known that an increase in external Ca concentration changes the surface-charge pattern and stabilizes the membrane as shown by shifts in voltage sensitivity. Several new mutants of Paramecium, teaB, a mutant P. tetraurelia, appears to have a more stabilized membrane. The curve of voltage sensitivity of the Ca channels (GCa-V relation) in teaB is about 10 mV more positive than that of wild type. The inactivation curve (max GCa-Vh relation) of the mutant is about 8 mV more positive than that of wild type. However, the mutant and the wild type have the same maximal GCa of 40 nmho/cell. The K conductance responsible for the anomalous rectification also appears more stabilized in the mutant.
981 CONDUCTANCE CHANGES ACCOMPANYING THE MATURATION OF MYOTUBES IN young unblocked membrane (Gm). In the present work tetraethylammonium Ca+ and Na+ conductance; and that the rise in membrane potential but that the decline in GC l was less than that observed for the diameters and values for specific membrane conductance were calculated by changes in the membrane conductance. The amine tested as cobalt salt included fluoride, chloride, acetate, citrate, and gluconate. There was no qualitative difference in the electrophysiological responses obtained with these amines. Nickel chloride and barium chloride were also able to elicit the periodic responses. The internal and external salt concentration could be varied independently without losing excitability. When the MnCl2 concentration in the external medium was reduced from 5 mM to 1 mM shorted the duration of the response and enhanced its amplitude. An effect similar to that of reducing the internal salt concentration could be obtained by increasing the inward current. Tetrodotoxin (2 µM) added to the external medium did not suppress the excitability. This simplest ionic condition achieved may help advance the physico-chemical theory of nerve excitation.

During maturation, the resting membrane potential of myotubes in culture increases over a period of several days from a low level (~10 mV) to that found in mature muscle fibres. Ionic conductances have been investigated during this time using a variety of methods. The use of single-salt solutions has been employed to study the effects of changes in the external salt concentration. Replacement of Na+, K+, and Cl- in the bathing solution by gluconate. There was no qualitative difference in the electrochemical profile of the chloride conductance. The anions tested as chloride and barium chloride were also able to elicit the periodic responses. The internal and external salt concentration could be varied independently without losing excitability. When the MnCl2 concentration in the external medium was reduced from 5 mM to 1 mM shortened the duration of the response and enhanced its amplitude. An effect similar to that of reducing the internal salt concentration could be obtained by increasing the inward current. Tetrodotoxin (2 µM) added to the external medium did not suppress the excitability. This simplest ionic condition achieved may help advance the physico-chemical theory of nerve excitation.

982 QUANTIFICATION OF ELECTROMAGNETIC FIELD STRENGTHS IN MEDICAL LINEAR ELECTRON ACCELERATORS AND THEIR IMPLICATIONS FOR PATIENTS WITH ARTIFICIAL CARDIAC PACEMAKERS. J. S. Jaeger* and T. F. A. Aminoglu, Department of Radiology Albert Einstein College of Medicine, Bronx, New York (10461) and Department of Physics Concordia University, Montreal, P.Q.

Behavioral changes in the neurophysiological responses of living organisms exposed to radiofrequency and microwave radiations have been well documented in the scientific literature. The character and intensity of these changes are dictated, among others, by the high sensitivity of many compartments of the CNS to such radiation exposures, the variability of electrophysiological properties of biological media, and the inherent characteristics of the ambient electromagnetic fields. Consequently, this vast array of interactive variables hampers the development of generalized but exact mathematical expressions to describe the many significant and novel bioeffects resulting from exposures to such wide energy spectrum. The specificity with which all pertinent parameters can be described may decrease the uncertainty in explaining the phenomenology of the effects both experimentally and theoretically. A unique biophysical problem with clinical implications involves individuals with artificial cardiac pacemakers exposed to pulse-modulated, 3000MHz, radiation emissions from medical linear electron accelerators. This work describes the experimental methodology employed in the quantification of the field gradients present in these facilities. A theoretical analysis will follow of the extraneous and induced electric field intensities in the chest walls of humans. The level of susceptibility and functional disturbances of representative models of pacemakers will be investigated for "free-field" and "simulated-implant" conditions. Preliminary test results will be discussed in connection with their clinical implications.

Ammonium rebound acidification (Boron and deMeer, J. gen. Physiol. 67: 91-112, 1976; Aickin and Thomas, J. Physiol. 273: 295-316, 1977) was used to lower intracellular pH of P. caudatum. Paramaecium, which normally exhibits regenerative but graded calcium responses (Naitoh, Eckert and Friedman, J. exp. Biol. 56: 667-681, 1972), produces all-or-none overshooting calcium action potentials under conditions of internal acidification.

The control bath solution consisted of 1mM CaCl2, 1.5mM KCl, 0.5mM KOH, 0.1mM EGTA and 1mM Hapes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer at pH 7.1. The ammonium solution consisted of the control saline to which NH4Cl was added to a concentration of 50mM. Specimens were bathed in the NH4Cl solution for two 15-minute periods separated by a 15-minute interval in the control saline. The paramecia were then returned to the control solution for intracellular current clamp or voltage clamp experiments. All-or-none activity in response to depolarizing current developed within 15 minutes after the final return to the control solution.

Hyperpolarizing and depolarizing current pulses of 200msec duration were passed to determine steady-state current-voltage relations. These relations were not altered from control values in those cells that were acidified and expressed all-or-none activity. The resting potentials of the acidified cells were also normal. In preliminary voltage clamp measurements the conversion to all-or-none behavior was not seen to be accompanied by any changes in the late currents. In contrast, conversion from graded to all-or-none activity as a result of lowered internal pH has been found in arthropod muscle to be associated with a reduction in delayed rectification (Woody, Soc. for Neuroscience Abst. 4: 236, 1978). The present experiments suggest that in Paramecium, lowered intracellular pH leads to all-or-none activity through an action other than an interference with late outward current. Supported by USPHS 1 T32 MH15345 and NSF BNS 77-19161.
MEMBRANE STRUCTURE AND FUNCTION
BENZODIAZEPINE AND GABA RECEPTORS IN NB2a NEUROBLASTOMA: ACTION ON Cl- FLOWS. E. K. Michaelis. Neurobiology Section, Dept. of Human Development, Univ. of Kansas, Lawrence, KS, 66045.

Grant GM 22357, and through Res. Service Award HD-07066 to the Kansas Center for Research in Mental Retardation.

In NB2a neuroblastoma cells as in brain the "GABA receptor unit" includes high affinity "g"-GABA and "h"-diazepam, "k"-clonazepam and "h"-flunitrazepam receptors. The thermostable endogeneous inhibitor of protein kinase (GABA-modulin) which inhibits both GABA and benzodiazepine binding is also present.

In these cells, as in brain, diazepam and GABA receptors appear to be functional. An expression of this function is that GABA (0.1 µM) increases the binding of [3H]-diazepam and [3H]-clonazepam, since in brain GABA receptors are thought to be coupled with Cl- ionophores we have tested whether also in NB2a cells the activation of GABA receptor with GABA or muscimol caused an increase in inward flux of 22Na. In presence of GABA receptor agonist a new steady state in the Cl distribution across NB2a membrane is obtained. The extent of this changes is related to GABA (10-5 to 10-1 M) or muscimol (10-5 to 10-4 M) concentrations. The increase in Cl- flux caused by GABA or muscimol is blocked by 10 µM bicuculline. In addition a dose of diazepam (10-4 M) which produces a release of [3H]-GABA from the cells but itself fails to change the Cl- flux, is capable of facilitating the increase in Cl- flux elicited by threshold doses of GABA, muscimol.

These results suggest that NB2a cells are a suitable model to study the interaction of benzodiazepine and GABA receptors at the molecular level.


The excitatory amino acid L-glutamic acid (Gl u) is known to produce its depolarizing effects through increases in Na+ conductance of neuronal membranes. Rat brain synaptosomes prepared in Picol1-sucrose gradients exhibited a rapid and a slow phase of 22Na uptake. This uptake process was enhanced by 0.5mM ouabain, K+ gradient and by L-Glu in a dose-dependent manner with maximal stimulation seen at 1µM L-Glu. This Glu-stimulated 22Na uptake was insensitive to at 1µM L-Glu. This Glu-stimulated 22Na uptake was insensitive to chair ^22Na in the 10-5-10-4M range were less by L-aspartic acid. This glutamate-induced 22Na uptake was insensitive to use at 10µM L-Glu. This Glu-stimulated 22Na uptake was insensitive to increase in specific STX binding activity. The purified STX binding protein, however, is not an Acanthus protein of about 9, comparable to that seen in solubilized but purified material. Further biochemical studies on the purified protein will be presented.


Antibody to the major coat protein (clathrin) of coated vesicles has been used to study the distribution of this protein in the nervous system. The coat protein was purified by SDS gel electrophoresis, extracted from the gel and used to immunize rabbits. The antibodies and immunofixation of polyacrylamide gels indicated the presence of specific IgG in the immune serum against the gel extract proteins. This antisera was used to study the distribution of coat protein in mouse cerebellum. Nerve and brain sections were examined. The aorta with a fixative containing 4.4% paraformaldehyde and 0.1% glutaraldehyde in 0.12M phosphate buffer. The cerebellum was stored overnight in phosphate buffered 4.0% paraformaldehyde and 40-50 µ slices were obtained for immunocytochemistry using a vibratome. The slices were incubated in rabbit preimmune or immune serum (1:2000 dilution) followed by peroxidase conjugated goat anti-rabbit IgG. After processing the slices for electron microscopic peroxidase cytochemistry, ultrathin sections were obtained and identified synaptical contacts on Purkinje cells and in cerebellar granule cells were studied. In these regions the labelling was highly concentrated in perisynaptic terminals of basket cell axons, mossy fiber axons and Golgi II axons. The antibody present in immune serum reacted with coated vesicles within these regions. These data suggest that either the coat protein contributes to structures within perisynaptic terminals other than coated vesicles or that these presynaptic terminals contain a pool of coat protein which takes part in the assembly and disassembly of coated vesicles.

Newly-synthesized (Na$^+$ + K$^+$)-ATPase (Na,K-ATPase) has been investigated in the holoenzyme (Na,K-ATPase) and the native cell membrane. The electroplaque membrane was isolated by a tissue dispersion technique. The enzyme was measured on the membrane using labeled Na$^+$ or K$^+$ and ATP.$^*$

Previously we reported a delay in incorporation of $^{3}H$ valine into the subunits purified from eel electroplaque membranes (Churchill and Hokin, Soc. for neurosci. abstr., 1977). The total cellular protein as analyzed by trichloroacetic acid precipitation did not show a 2 to 3 h delay in incorporation. Therefore, the delay could not be explained by slow equilibration of the amino acid precursor. Further evidence that a lag in appearance of newly-synthesized Na,K-ATPase exists is presented.

After exposure to the protein synthesis inhibitor, cycloheximide, the holoenzyme no longer incorporates $^{3}H$ valine into its large and small subunits. This lag probably represents post-translational events which occur prior to entry into the plasma membrane.


Some identified neurons in the visceral ganglion of Aplysia californica contain a membrane system which gives rise to slow relaxation oscillations of membrane potential. These oscillations were investigated by conventional voltage clamp. Steady state and dynamic I-V-characteristics, current relaxation times, and membrane capacity were measured using step and ramp commands in different ionic concentrations of the extracellular solution. The shape of I-V diagrams consisting of the single steadystate of dynamic I-V-characteristics was found to give the criterion for the stability of the membrane potential at a constant net current.

Changes of the K$^{+}$concentration position and slope of the steady state I-V-characteristics are in accordance with the constant field theory at inward currents, while at zero currents they are not. I-V-diagrams undergo transformations which can be described as contractions in the direction of the voltage axis with increase of K$^{+}$concentration and as expansions with decrease of K$^{+}$concentration.

1 Ca$^{2+}$cells tend to start oscillating and oscillating ones tend to become bistable. The transference of I-V-diagrams caused by reconstitution of the Na$^{+}$concentration and the relaxation of dynamic I-V-characteristics in the direction of the voltage axis.

In control solution a reversal potential of the relaxed currents could not be found between -80 mV and -20 mV. Reduction of the Na$^{+}$concentration strongly decreases the amplitude of the relaxing currents.

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ARRANGEMENT OF MULTIPLE MONOAMINE OXIDASE ENZYMES ACROSS THE OUTER MEMBRANE OF RAT BRAIN MITOCHONDRIA. Robert Faulkner* and Rosa Huang* (SPOR: J-Y Ma). University of South Alabama, Mobile, Alabama 36688.

Phospholipases C and D (LC, LD) were used to remove the surface head groups of membrane phospholipids of an intact, purified rat brain mitochondrial preparation. Both LC and LD treatment under conditions of varying lipase concentration and incubation time attacked only phosphatidylycholine (PC) and phosphatidylethanolamine (PE). PC and PE constituted about 80% of the brain mitochondrial phospholipids. After lipase treatment, the bilayer structure of the outer mitochondrial membrane was retained. In the case of LC treatment, MAO-B sites were inactivated to a much greater extent than MAO-A sites. MAO-B sites were more peripheral. MAO-A sites were located below the alcohol moiety of the membrane phospholipids. LC treatment eliminated the phosphoryl alcohols and thus exposed a few Å deeper into the bilayer midline in contrast to the action of LD. The data indicated that MAO-A sites then became accessible. Further, an additional class of MAO-B sites was exposed by the action of LC. (Supported by NIH Grant #NS-144434)


Quinacrine was found to bind specifically to the axolemma (axonal membrane) of bovine corpus callosum. We have studied this binding reaction by monitoring fluorescence changes of quinacrine. Local anesthetics compete for binding at equilibrium and incubation time attacked only phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PC and PE constituted about 80% of the brain mitochondrial phospholipids. After lipase treatment, the bilayer structure of the outer mitochondrial membrane was retained. In the case of LD treatment, MAO-B sites were inactivated to a much greater extent than MAO-A sites. MAO-B sites were more peripheral. MAO-A sites were located below the alcohol moiety of the membrane phospholipids. LC treatment eliminated the phosphoryl alcohols and thus exposed a few Å deeper into the bilayer midline in contrast to the action of LD. The data indicated that MAO-A sites then became accessible. Further, an additional class of MAO-B sites was exposed by the action of LC. (Supported by NIH Grant #NS-144434)


Explant culture of the adult goldfish retina results in vigorous neurite outgrowth, provided that the optic nerve has been crushed in vivo 18-24 h prior to explantation. We have used this preparation, coupled with a direct membrane marker,.Concanavalin A (Conn A) to determine the site of new membrane addition in growing neurites. We had previously determined that Con A labels neurite cell surfaces. In order for Conn A to be a useful marker for studies of membrane addition during neurite outgrowth, it was necessary to determine conditions under which the lectin would not be toxic to the culture (i.e., prevent neurite outgrowth) and also would not diffuse into the new areas of growth. Initial studies indicated that high concentrations of Conn A were toxic to the culture while lower doses of Conn A, which were nontoxic, diffused rapidly into new areas of growth. This low dose of Conn A (10 µg/ml) could be immobilized by the subsequent addition of 100 µg/ml of lectin antibody (anti-Con A). This treatment did not prevent growth. Furthermore, the receptor/Conn A/anti-Con A complex was not appreciably internalized, making it an ideal membrane marker for the following experiments.

A 3 d culture was labeled with 10 µg/ml Con A. The neurites were then tagged with 100 µg/ml rabbit antibodies to Conn A, washed, and left to grow in medium. After 24 h, the neurites were labeled with fluorescent goat anti-rabbit antibodies. Thus, only old membrane that had been tagged 24 h previously with Conn A/anti-Con A would now bind the fluorescent antibodies. On the other hand, any membrane that had been added during the 24 h interval would not have the Conn A/anti-Con A complex on its surface and, consequently, would not bind the fluorescent antibodies.

Using this paradigm, we observe bright, uninterrupted fluorescence of old membrane, while the entire extent of new membrane, including the growth cone, is unlabeled. These results are in agreement with previous studies of Gray (PNAS 66:906-910, 1970), in which inert particles served as stationary markers during growth of cultured rat sympathetic neurons. In the present studies we have used a more direct membrane marker to indicate that the locus of new membrane addition is at the growing end of the neurite. We believe that, during growth, membrane components are transported via axonal flow and fuse with preexisting membrane in the region of the growth cone.

K+ EFFECT ON GLIAL AND PERIKARIAL (ILK+K+) ATPase FROM NORMAL AND PATHOLOGICAL HUMAN BRAIN. Thierry Grisar*, George Franck*, and A.V. Delgado-Escueta. Dept. of Neurology, Reed Neurological Research Center, Los Angeles, California 90024 and Dept. of Neurology, University of Liege, Belgium.

The role of glial cells in the active control of extracellular potassium (K+) has been documented in animals. This is particularly true when considering the sensitivity of the glial (ILK+K+) ATPase to K+ (Grisar et al. Brain Res. 1979).

A first attempt in this field is now available in human brains. The effect of K+ on ILK+K+ ATPase activity was determined in cell fractions obtained from human brains. The enzyme activity, ultracentrifugation on discontinuous Sucrose-Ficoll gradient from 3 "normal" patients. As in rabbit, the glial fractions exhibit a higher level of enzyme activity than the neuronal fraction. This may be explained by a higher content of membrane protein in glial fractions. However, like in the animals, glial enzyme is markedly activated by K+ ions between 5 to 20 mmo while this phenomena is not observed in perikarial fractions. These findings in human brains confirm the observations in animals on the role of glial cells. These "control" brains were compared with those above from one case of psychosis, one case of Alzheimer Disease and one case of Creutzfeld Jacobs Disease (CJD). In the two first cases, enzyme activities were decreased in both fractions while the K+ ion sensitivity of glial enzyme was not observed. These results will be due to the delay between death and autopsy as well as the technical procedure used (i.e., the low sensitivity to K+ in these glial cells remain unexplained. More studies are needed. In CJD no significant change was found. These latter findings do not support Bignami and Palladini who hypothesized a defect in kinase enzymes in CJD.

Reconstitution of a putative neurotransmitter receptor protein into a liposome with recovery of ionomycin function is crucial to the characterization of the protein as the physiologic receptor. The present study represents the first attempt to reconstitute the presumed glutamate receptor protein purified from rat brain synaptic membranes into liposomes of varying phospholipid composition by means of equilibrium dialysis or simple room temperature incubation (Racker, 1976).

The glutamate (Glu) binding protein (GBP) was isolated as previously described (Michaelis, 1975). The concentrated GBP was then dialyzed extensively against 50 mM Tris-Cl buffer, pH 7.4, following a 30 minute incubation at room temperature, a mixture of GBP and phosphatidyl-serine (total volume 200 l) was loaded onto a Sepharose 6B column and eluted with 50 mM Tris buffer.

Preliminary evidence indicates that a high molecular weight aggregate of GBP which eluted in the early fractions exhibited high specific activity of L-(M)-Glu binding. The aggregate also exhibited high affinity binding for (M)-kainic acid, which is of interest in light of previous studies from this laboratory indicating that the solubilized, non-aggregated GBP does not bind (M)-kainic acid (Kuonen and Michaelis, 1979).

Several fractions immediately following the GBP aggregate showed little or no L-(M)-Glu binding when tested on the same day as the reconstitution. When these fractions were tested five days later, they bound considerably more L-(M)-Glu than the higher molecular weight GBP aggregate. These fractions contained a stable amount of the phosphatidyl-serine and appear to represent the reconstituted system. Studies are currently underway to determine whether the time delay between reconstitution and the appearance of L-(M)-Glu binding in these fractions is due to the co-elution of soluble GBP and liposomes which then form a proteoliposome reconstituted system. This increased binding may also be due to the rearrangement of the GBP in the lipid matrix from an initial low-binding to a higher specific binding conformation.

This research was partially supported by DHM Res. Serv. Award HD 07064 from NIMH to the Kansas Center for RS & HD, by a grant from NIGMS, GM 22357, and by Biomed. Res. Support Grant 50706.

DIFFERENT EFFECTS OF TEA VS. 4-AP AND 3,4-DAP ON THE CALCIUM INWARD CURRENT SYSTEM IN APYLSIA NEURONS. Manfred R. Kleie* and Yoshimi Ikemoto* (SPON: John M. Servey). MPI Brain Res., Dept. Neurobiol., Deutschordenstr. 46, D-6000 Frankfurt-M., FRG.

4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP) have been shown to block potassium channels in the squid axon membrane extracellularly, whereas tetraethylammonium (TEA) acts internally; 3,4-DAP was about 50 times more potent than 4-AP. Two types of neurons can be distinguished within the visceral ganglion of Aplysia with respect to the action extracellular TEA and 4-AP have on their action potentials (APs) and membrane currents (Kleie, JP, 26:125F, 1978). To those data the following results were added: i) F cells possess a slow, non-inactivating, calcium-dependent inward current (I_{Ca2^+}), which is about 1/10 of that seen in S cells (approx. 20 nA). ii) 4-AP and 3,4-DAP with nearly equal potency delay and reduce the outward current in both F and S cells. At the same time the duration of APs is increased but never to that of plateau spikes, unless TEA is added. iii) The most important difference between TEA vs. 4-AP and 3,4-DAP is the effect the latter have on I_{Ca2^+}. Even in concentrations which have very little effect on the outward current (100-600 µM), both substances can increase I_{Ca2^+} by 100% in contrast to TEA, which produces no increase. The increase of I_{Ca2^+} could explain why in those cells having small I_{Ca2^+} (i.e. F cells) TEA can induce calcium-dependent plateau spikes only in the presence of 4-AP or 3,4-DAP.

FREEZE-FRACTURE OF MUSCLE SPINDLES. William R. Kennedy, Donald C. Quick* and Thomas S. Reese. Departments of Neurology and Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455; and Section on Functional Neuroanatomy, NINCDS, National Institutes of Health, Bethesda, MD 20014.

Isolated muscle spindles from hamsters, cats, and rabbits were freeze-fractured by conventional methods. Prominent features of the capsule are numerous vacuolizations and the presence of occluding junctions. The junctions are extensive, in accordance with the known impermeability of the capsule (Kennedy and Yoon, in press, Muscle & Nerve). Occluding junctions are composed of rows of fused particles associated with the protoplasmic (P) fracture faces. Similar, less extensive junctions are seen in the capsule cells immediately surrounding the intrafusal muscle fibers.

No communicating junctions were observed, indicating that they are rare or absent.

Rectilinear arrays of P-face particles were seen on the spindle muscle fibers; their appearance is identical to those found on extrafusal muscle. The arrays on intrafusal muscle fibers are concentrated in areas tentatively identified as post-synaptic membranes of the 'trail' type motor endings. Rectilinear arrays can be seen occasionally on other parts of the muscle fiber surface, but there are few or none in the sensory zones.

Adhering junctions and invaginations (Kennedy, Webster and Yoon, 1975, J. Neurocytol. 4:675) have been observed in the membranes of sensory endings.


The presence of filamentous proteins in close association with synaptic plasma membranes (SPM) now appears well established on the basis of both biochemical and morphological studies. Tubulin, filamin, myosin, actin, tropomyosin, calmodulin and perhaps some of the subunits of troponin all have been claimed to form part of the SPM. Synaptosomes, SPM, synaptosomal junctional complexes, post-synaptic densities and post-synaptic membranes have been isolated and analysed for the presence of the above mentioned proteins both qualitatively and quantitatively. The membrane proteins were identified and interrelationships established by comparison to purified reference proteins using the techniques of one-dimensional fingerprinting and two-dimensional gel electrophoresis (isoelectric focusing and SDS-polyacrylamide electrophoresis). (Supported by Research Grant NS 08309 from the NIH)
1004 DIFFERENT EFFICACIES OF \( \beta \)-AND \( \gamma \)-AMINO-\( \beta \)-HYDROXYBUTYRIC ACID FOR GABA RECEPTOR-RELATED BINDING AND CONDUCTANCE INCREASES.
Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010 and "Institute for Neurobiology, Okayama University Medical School, Okayama, Japan.

The structure of the GABA receptor was investigated by determining the relative effects of two stereoisomers of the GABA agonist, \( \gamma \)-amino-\( \beta \)-hydroxybutyric acid (GABOB), in several quantitave receptor-related assay systems. \( \gamma \)GABA was found to be about twice as potent as \( \beta \)GABOB. d-GABOB was found to be again more potent than \( \beta \)GABOB in mimicking the postsynaptic action of GABA, which was measured as increases in membrane input conductance in the isolated crayfish stretch receptor neuron. Both GABOB enantiomers have some affinity for GABA transport processes, and d-GABOB was found to be more potent than \( \beta \)GABOB in inhibiting GABA uptake into rat brain synaptosomes and Na\(^+\)-dependent GABA binding to mouse brain membranes. For this reason, conductance measurements also were made in the presence of \( 10^{-5} \)M nipeucetic acid or L-O-diaminopropionic acid, two specific GABA transport blockers. However, \( \beta \)GABOB was again more potent than d-GABOB. The order of potency in the receptor-related binding assays (d-GABOB=\( \beta \)GABOB) could not be altered by detergent treatment, changes in buffer composition, or by preparing the tissue at room temperature. The greater effectiveness of \( \beta \)GABOB in the physiological assay does not appear to be due to species differences. \( \beta \)GABOB, but not d-GABOB, is an effective inhibitor of induced seizure activity in cat brain (Y. Katayama and A. Mori 1977) and motor cortex (H. Aihara, A. Akimoto, T. Makita 1976). Thus, d-GABOB is more potent than \( \beta \)GABOB in in vivo systems. While the reasons for this discrepancy are unclear, these findings may suggest that the structural asymmetry of the GABA recognition site in vitro is somewhat different than that related to activation of GABA recep

1005 ROLE OF PHOSPHORYLATION ON GABA RECEPTOR ACTIVITY.
A. Leon*, A. Guidotti, G. Toffano and E. Costa.

"GABA-modulin", a thermostable, acidic (M.W. 15,000 dalton) protein isolated from brain, non-competitively inhibits the Na\(^+\)-dependent high affinity uptake of GABA to synaptic function membranes. Brain preparations of this inhibitor block competitively cyclic AMP-independent protein kinase in supernatant of brain homogenates. The high affinity binding of \( ^{3} \)H-muscimol to the GABA recognition site of postsynaptic GABA receptor-anionophore complexes. A similar order of potency for the GABOB enantiomers was observed for the cerebrovascular GABA receptor recently characterized in our laboratory with \( ^{3} \)Hmuscimol binding assays using bovine cerebral blood vessels. In contrast to the binding results, \( \beta \)GABOB was significantly more potent than d-GABOB in mimicking the postsynaptic action of GABA, which was measured as increases in membrane input conductance in the isolated crayfish stretch receptor neuron. Both GABOB enantiomers have some affinity for GABA transport processes, and d-GABOB was found to be more potent than \( \beta \)GABOB in inhibiting GABA uptake into rat brain synaptosomes and Na\(^+\)-dependent GABA binding to mouse brain membranes. For this reason, conductance measurements also were made in the presence of \( 10^{-5} \)M nipeucetic acid or L-O-diaminopropionic acid, two specific GABA transport blockers. However, \( \beta \)GABOB was again more potent than d-GABOB. The order of potency in the receptor-related binding assays (d-GABOB=\( \beta \)GABOB) could not be altered by detergent treatment, changes in buffer composition, or by preparing the tissue at room temperature. The greater effectiveness of \( \beta \)GABOB in the physiological assay does not appear to be due to species differences. \( \beta \)GABOB, but not d-GABOB, is an effective inhibitor of induced seizure activity in cat brain (Y. Katayama and A. Mori 1977) and motor cortex (H. Aihara, A. Akimoto, T. Makita 1976). Thus, d-GABOB is more potent than \( \beta \)GABOB in in vivo systems. While the reasons for this discrepancy are unclear, these findings may suggest that the structural asymmetry of the GABA recognition site in vitro is somewhat different than that related to activation of GABA recep...
LIPID-PROTEIN INTERACTIONS IN MEMBRANES CONTAINING THE ACETYLCHOLINE RECEPTOR (AChR) is reversibly phosphorylated by a protein kinase. The acetylcholine receptor (AChR) in membrane vesicles prepared from mammalian brain, target muscle and synaptic membranes contains a M.W. of 41,000, whose phosphorylation was stimulated approximately 4-fold by KCl (25-100 mM). This effect was not seen with NaCl. Both Ca²⁺ and Mg²⁺ were effective at 10⁻⁴ M and had no effect on phosphorylation of the 41,000 M.W. protein.

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Several lines of evidence suggest that the 41,000 M.W. phosphorylated polypeptide may be the AChR. The acetylcholine binding subunit of the AChR in membranes from mammalian brain, target muscle and synaptic membranes contains a M.W. of 40,000. The AChR for fetal calf myotubes in culture is the same as that reported here (Nerl et al, JBC 253:2861, 1978). The approximate molecular weight of the AChR in myotubes was estimated to be 41,000 by polyacrylamide gel electrophoresis. In contrast to the results with myotubes, membranes prepared from myoblasts did not show potassium-stimulated phosphorylation of a 41,000 M.W. polypeptide.

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The triad structure from skeletal muscle has been isolated by a modification of a procedure previously described by Caswell et al. (Arch. Bioch. Biophys. 194: 417, 1979). To facilitate the isolation of the triad components (Ca++ ATPase and pumping, adenylate cyclase, and H-ouabain binding). The acetylcholinesterase specific activity in the triad fraction is approximately 60 nmoles/mg-min. Specificity for acetylcholinesterase can be shown both by butyrylcholine and acetylcholine as substrates and by inhibition studies using the ACHE inhibitor, BW 284c51, which decreases acetylcholinesterase activity in a light density fraction (equivalent to 25% sucrose density) and co-migrating with non-purified preparations. Additional aliquots were incubated with cholesterol/phosphatidylcholine vesicles containing a 2:1 molar ratio of cholesterol to egg yolk phosphatidylcholine. Synaptosomes were isolated from rat forebrain by differential centrifugation and were found to be enriched in the cholesterol/phosphatidylcholine co-dispersions. Following the incubation, the synaptosomes were collected by centrifugation and washed. Lipids were extracted for cholesterol and phosphorous determination. A three hour incubation at 32°C resulted, for an excess of brain toxin-binding site complexes were incubated with dilutions of anti-ACH receptor (received from M. Elderfield) for 0-8 hrs at 4 or 2°C. An excess of goat anti-rabbit IgG (GAR) was added and the samples incubated for 0-24 hrs and centrifuged at 2,000 rpm for 10-240 min. The maximum precipitation was 10% (we obtain 1-2% with non-purified preparations). Additional aliquots were incubated with an excess of anti-ACH receptor, as above, passed down Sepharose 4B or 6B gel columns and collected in 3 or 5 ml fractions. Each fraction was analyzed for radioactivity and the specific activity of antibody was confirmed by immunoprecipitation. The results clearly indicate a depletable peak of antibody associated sites; the number of sites is identical to that found by the standard immunoprecipitation procedure.

In other studies, we have observed that antisera prepared against Butox will precipitate 1-2% of the sites. Furthermore, anti-ACH receptor may not precipitate immunoprecipitated anti-Butx.

The validity of our methods has been shown by carrying out these procedures using purified electric fish and denervated nerve terminals, thus regulating the release of neurotransmitter. We are currently favor the hypothesis that nAChR from different cellular populations may have different antigenic determinants.

This research is supported by grants BNS 78-13724 and BNS 78-23604 to Barbara J. Horley and N1 14262 to George E. Kemp.
EFFECT OF VASOPRESSIN ON THE PENETRATION OF 14C-UREA INTO BRAIN COMPARTMENTS PROTECTED BY BARRIER SYSTEMS. Z. Paranovský* and C.E. Johanson* (SPON: E.C. Beck). Dept. of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah 84132.

Vasopressin increases the permeability of some epithelia to urea. Since the choroidal epithelium is relatively impermeable to urea, it is conceivable that vasopressin could alter the permeability of the choroidal epithelium (perhaps the cerebellum capillaries) to urea. Using in vitro as well as in vivo preparations, we have investigated the effects of various doses of vasopressin on the uptake of radioisotopes by tissues protected by the blood-CSF barrier (choroidal plexus) and the blood-brain barrier (cerebellum and cerebral cortex).

Lateral ventricle choroid plexus (LVCP) from adult rats was incubated in artificial CSF with or without vasopressin (100 ml/ml); plexus tissue from one lateral ventricle served as control for tissue taken from the contra-lateral ventricle.

### 14C-urea spaces in LVCP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>14C-urea spaces in LVCP</th>
<th>pCO₂</th>
<th>pH</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.5 ± 3.1</td>
<td>37.4</td>
<td>7.4</td>
<td>64.2 ± 3.8</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>7.4 ± 0.2</td>
<td>37.4</td>
<td>7.4</td>
<td>64.2 ± 3.8</td>
</tr>
</tbody>
</table>

Measurements were made of "periodicity" as defined by the cycle times between response periods and off periods. Periodicity was a function of both the frequency and magnitude (pulse current X pulse duration) of the stimuli. Our results matched with periodicity-frequency and periodicity-magnitude relations obtained from a numerical model of the threshold curves subjected to similar conditions of stimulation. The activity-dependence of threshold appears to be sufficient to account for intermittent responsiveness.

Redistribution of Na⁺ and K⁺ IN RAT CNS DURING RESPIRATORY ACIDOSIS AND ALKALOSIS. Lynn K. Peschling* and C.E. Johanson* (SPON: E.C. Beck). Dept. of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah 84132.

Acute disturbances of acid-base metabolism can profoundly alter the distribution of Na⁺ and K⁺ in some tissues. To evaluate the separate influences of plasma [HCO₃⁻] and [Na⁺] on Na⁺ and K⁺ distribution in CNS tissues, previous studies involving metabolic acid-base disturbances were extended to an intravenous infusion of vasopressin (Fed. Proc. 38: 375, 1979). Anesthetized (150 mg ketamine/g) Sprague-Dawley rats (400-500 g) were tracheotomized and norrerventilated on a rodent respirator with air (control) or 5% CO₂ (acidosis), or hyperventilated with air (alkalosis). Animals were sacrificed by exsanguination at 1 hr, at which time blood from the abdominal aorta was analyzed for pH and pCO₂.

### Treatment

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<tr>
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<td>64.2 ± 3.8</td>
</tr>
<tr>
<td>Alkalosis</td>
<td>26.8± 0.9</td>
<td>7.4</td>
<td>64.2 ± 3.8</td>
</tr>
<tr>
<td>Acidosis</td>
<td>26.8± 0.9</td>
<td>7.4</td>
<td>64.2 ± 3.8</td>
</tr>
</tbody>
</table>

### Changes in CSF and plasma electrolytes

While significant changes in biochemical constituents of CSF and plasma were observed, there were no significant changes in electrolyte concentrations. In contrast, internal CSF electrolyte levels were significantly altered by vasopressin.

Recent studies suggested the existence of bungarotoxin (α-Butox) binding sites in the goldfish tectum. However, it is not yet clear whether these sites are presynaptic or postsynaptic. The present fluorescent microscopic studies support evidence for the presence of presynaptic α-Butox binding. Binding of rhodamine-conjugated α-Butox (R-α-Butox) was observed on both optic nerve sections and on neurites of cultured retinas explanted from adult goldfish whose optic nerve had been crushed 10-14 d previously. Optic nerve sections were obtained from goldfish and were fixed in a mixture of ethanol, formaldehyde and acetic acid. R-α-Butox binding to cultures fixed under mild conditions was high, while the binding was low in vivo. In both nerve sections and neurites in culture, preincubation with 10^-7M unlabeled α-Butox blocked the binding of 10^-10 M R-α-Butox. The pharmacological profile of R-α-Butox binding to the neurites in culture was investigated in the presence of neurotropic drugs. D-α SAPS-substrate and carbachol were ineffective in inhibiting the binding while atropine was ineffective. These experiments have shown that α-Butox binding is present in optic nerve axons and the in vivo results indicate that re-establishment of synaptic connections is not necessary for its presence. Both in vivo and in vitro, axonal sites are not accessible from the exterior of the cell. Presynaptic α-Butox binding sites may play a role other than in events related to neurotransmission.


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High-affinity sodium-dependent transport of taurine by a glomia cell line (LLM 55) has been described by Shain and Seligmann (Res. Chem., in press). Since sodium-dependent transport mechanisms for neutral amino acids may involve the cotransport of sodium with the substrate, they are potential candidates for LLM 55. We have therefore investigated the relationship between taurine transport and membrane potential in LLM 55 cells.

Membrane potential was measured by intracellular recording and also calculated from the distribution of the tritiated molecular probe triphenylmethylphosphonium ion (TPMP+). The average recorded membrane potential was -43 ± 3 mV and decreased to -63 mV in normal potassium solutions and made the dependence of membrane potential on external potassium more nearly Nernstian. Ouabain (10^-7 M) had no significant effects. However, if cells were treated for 30 min with ouabain and then exposed to taurine, a depolarization was observed.

TPMP+ required 60 s to equilibrate across the membrane. The dependence of TPMP+ distribution on potassium was similar to the recorded membrane potential, but the absolute calculated values were substantially greater. The membrane potential was measured in normal- and low-sodium solutions. Depolarization with potassium in normal-sodium solution had no significant effects. The initial rate of taurine uptake, nor did hyperpolarization with valinomycin. In low-sodium solutions, increasing potassium inhibited the initial rate of uptake but valinomycin still had no significant effects. Ouabain increased the amount of TPMP+ accumulated by cells in agreement with its effect on the intracellularly recorded membrane potential. However, control experiments showed that the low-TPMP+ accumulated by cells, which was not consistent with intracellular recordings. Because of the kinetics of the TPMP+ distribution and the large amount of membrane insensitive to TPMP+, we conclude that this probe is of limited value in this cell type.

The effect of membrane potential on taurine transport was measured in normal- and low-sodium solutions. Depolarization with potassium in normal-sodium solution had no significant effects. The initial rate of taurine uptake, nor did hyperpolarization with valinomycin. In low-sodium solutions, increasing potassium inhibited the initial rate of uptake but valinomycin still had no significant effects. Ouabain increased the amount of TPMP+ accumulated by cells in agreement with its effect on the intracellularly recorded membrane potential. However, control experiments showed that the low-TPMP+ accumulated by cells, which was not consistent with intracellular recordings. Because of the kinetics of the TPMP+ distribution and the large amount of membrane insensitive to TPMP+, we conclude that this probe is of limited value in this system.

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Using freeze-fracture techniques, we have analyzed the glial axonal junction (G-A-J) between Schwann cells and axons in the peripheral nervous system, and oligodendrocytes and axons in the central nervous system. We have identified a new set of dimeric-particles arranged in circumferential rows within protoplasmic fracture faces (P-faces) of the paranodal axolemma in the region of glial-axonal juxtaposition. These particles were observed in both aldehyde-fixed and non-fixed preparations. The dimeric-particles are 260 Å in length, composed of two 115 Å subunits. The rows of dimeric-particles within the axonal P-face leave complementary rows of pits within the external fracture face (E-face) of the paranodal axolemma. These axonal particles are positioned between rows of 160 Å particles occurring in both fracture faces of the glial loops in the region of the G-A-J. In addition to these previously described 160 Å particles, we observed a new set of 75 Å particles within the glial P-faces of the G-A-J. These 75 Å glial particles form rows that are centered between the rows of 160 Å particles and therefore are superimposed over the rows of dimeric-particles within the paranodal axolemma. Our new findings are interpreted with respect to methods of specimen preparation as well as a potential role for the paranodal organ in saltatory conduction. We conclude that this particle rich junction between axon and glia could potentially provide an intricate system of ion exchange between the two cells.

[Supported by grants to M. Ellisman from MDA and NIH #NS14718; and C. Wiley from NEI/NIH training grant PHS #E007198.]
MEMORY AND LEARNING
PUROMYCIN DOES NOT OBLITERATE ALL THE DIFFERENT MEMORIES WHICH OCCUR IN A GIVEN TRAINING SITUATION. D.A. Barraco, E.M. Eisenstein, and K.L. Lovell. Departments of Physiology and Biophysics and the Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

A new one-session training procedure for cockroaches, in which animals are trained to turn right or left to avoid a shock has been developed. This paradigm was utilized to test the effects of puromycin, a protein synthesis inhibitor, on learning and memory. The drug was injected before training in doses causing approximately 70% protein synthesis inhibition. The number of correct turns and the time taken for the animal to proceed down the runway were the two behavioral parameters measured in the paradigm. In control animals the number of correct choices and the runway time both increased with succeeding trails during training. In addition, control animals showed excellent retention of these responses 5 hours later during testing (n=23). Puromycin also had no effect on acquisition of the two responses. However, upon testing, experimental animals showed a significant retention deficit of the correct turn response but not of the behavioral modification evidenced by increased runway time (n=31). Thus, puromycin may provide specificity for the different types of behavioral plasticity that occur in any training situation and that may be mediated at different levels of the nervous system. (Support in part by the Biomedical Research Support Grant to the College of Natural Science).


Young chicks were trained to suppress their tendency to peck a small bead after it had been dipped in a noxious substance, methyl anthranilate (MeA). Retention was measured by the proportion of a group of chicks pecking an identical clean target. Chicks were able to discriminate between the previously tainted bead and a novel one of different colour, for up to 24 hr after the learning trial. There was good interocular transfer for the task when chicks were trained and tested monocularly.

Recent work with this task using intracranial injection of ouabain or cycloheximide (Bell and Gibbs, Neurosci.Lett., 1979, in press) suggests that monocular learning results in a local- enzised engram in the forebrain hemisphere contralateral to the trained eye. Interocular transfer of the MeA task in the intact chick therefore requires interhemispheric readout of the unilateral engram by the trained hemisphere. A bilateral engram can become established as a consequence of interocular transfer testing; the readiness of engram duplication depends on the recency of formation of the original trace.

We lesioned the dorsal supra-optic commissure (DSO) in three day old chicks 4 hr after monocular learning of MeA-avoidance, and following 48 hr of monocular exposure to an imprinting object. A sham operated control group was taken from the same hatch, similarly housed and trained.

At 24 hr after operation the sham group (n=15) showed good retention of the MeA task with the trained and untrained eyes, and discrimination between the previously tainted target and a novel one. The split-brain group (n=25) showed good retention and discrimination with the trained eye. When tested with the untrained eye, chicks failed to show retention and pecked the learning and novel targets with the same probability. This suggested that monocular learning of MeA-aversion established a lateralized engram and that retention is to it depends on an intact DSO.

Interocular transfer of imprinting failed in the split-brain and sham groups after operation, but succeeded in some individual chicks from both groups, on three subsequent testing days. It appears that imprinting is more sensitive to averse reversal than the MeA task. Further work is required to resolve whether monocular imprinting establishes a bilateral engram or whether retrieval of a unilateral engram is achieved independ-ently of the DSO.


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Commonly, studies on the acquisition of instrumental behaviors involve "shaping" of the animal's responses by the experimenter. Thus, acquisition becomes the result of an interaction between the animal's learning capabilities and the experimenter's ability to shape, autoshaping procedures, on the other hand, represent an important technical advancement with respect to the method of successive approximations, since the behavior of the animals may change within a standardized experimental environment, following a direct intervention of the experimenter. We studied the effects of cholinergic blockade of the caudate nucleus on the acquisition of lever pressing behavior. Animals were trained in a Skinner box on day 1, a light-bulb situated above a centrally located liquid dispenser was turned on and then 0.3 ml of water were delivered. This sequence was repeated 50 times with an intertrial interval (ITI) of 30 sec. Twenty-four hr later a light-bulb placed above a lever was turned on 50 times (ITI 30 sec). If during these periods a rat pressed the lever the liquid dispenser was lighted and water was delivered. If the animal pressed while the lever was not lighted, the response was not reinforced. Bilateral microinjections of scopolamine (30 µg into each cannula) or saline solution (NaCl) were performed in independent groups 6 min before the second session, into the anterior caudate nucleus (ACN), the posterior caudate (PCH) or into the parietal cortex (Ctx); there was also an unplanted (G1) control group. Those groups injected with scopolamine in ACN and PCH showed an impairment in the acquisition of lever pressing, as compared with the Ctx, NaCl and unplanted groups. These results suggest that cholinergic activity of the CN is involved in the acquisition of instrumental behaviors.


We recently reported that in a conditioning paradigm which elicited a number of behavioral responses, densely distributed conditioned factors, a proportion of neurons in medial geniculate of rat showed short latency associative change. The present experiment was designed to determine if such units existed and to study the relationship of the subdivisions of the medial geniculate to and identify response characteristics which might differentiate these units from units not showing associative change. The multiple unit responses on 117 probes distributed throughout Mn were recorded during differential appetitive conditioning, extinction, and the subsequent reversal. The responses were characterized by the use of response net criteria for responsiveness and stability of recording and were further analyzed. 11 units which showed enhanced response to the positive stimulus relative to the response to the negative stimulus throughout or more consecutive reversals were considered to be associative. These units were largely localized in the far posterior portion of Mn where 84% (9 of 36) units were associative. Only 5% (2 of 37) units in the remainder of Mn were associative. Associative units were further differentiated from non-associative units by differences in the temporal pattern of their initial response prior to conditioning. Units with a more sustained response were more likely to show associative change than those with a very transient response and the magnitude of change was positively related to the initial duration of response. 71% (5 of 7) of units which initially were responsive for more than 80 ms showed large magnitude associative change during conditioning. Only 9% (6 of 66) of units which were initially responsive for 50 ms or less showed associative changes and these were of small magnitude.

These associative neural changes were shown not to result from behavioral feedback since their latency (as early as 16 ms after stimulus onset) preceded behavioral response and since they were not directly correlated. They appear more directly related to processing of the significance of stimulus information. The fact that these changes are localized to a discrete portion of Mn with known differential afferents and efferents and that associative units have different temporal response patterns which are easily identified leads to the potential for identifying the neural circuitry involved using single unit analysis techniques. (Supported by NSF BNS 77-22059)

1032 CHANGES IN RESPONSIVENESS TO GLABELLA TAP AMONG NEURONS IN THE SENSORIMOTOR CORTEX OF AWAKE CATS. J. Brons* and C.D. Woody. Dept. Anatomy & Psychiatry, Brain Research Institute, Mental Retardation Research Center, UCLA Medical Center, Los Angeles, CA 90024.

Unit responses to glabella tap stimulation were studied in 120 neurons of the sensorimotor cortex in 4 awake cats. The glabella tap, used as an unconditioned stimulus (US) during classical conditioning, elicited an unconditioned eyelink response of 7-9 msec latency by activation of a brainstem reflex arc (Wood & Brozek, J. Neurophysiol. 32: 704-716, 1969). The effects of the tap US on cortical responsiveness were examined over a two day period (up to 400 tap trials per day, 5 sec ITI). Overt habituation of the eyelink response occurs at ITI's of 1-2 sec or less.

Unit activity was recorded intracellularly or extracellularly with 15-50 MΩ, 1.4M K+-citrate filled glass microelectrodes. On the first day of recording, out of 55 neurons, 29% showed increased activity to tap; 36% showed decreased activity; and 35% were unresponsive. On the second day, out of 73 units, 55% showed increased activity, 27% decreased activity, and 18% were unresponsive. Analysis indicated that the proportion of cells responding with increased activity had increased significantly from the first day to the second (P<0.01, 2-tailed t-test). PST histograms of the responses to the tap revealed that the mean onset latencies (9 msec for increased activity, 13 msec for decreased activity) and peak amplitudes of the increased unit responses remained unaltered over the two days. Intracellular recordings indicated that the increases and decreases in evoked activity were supported by EPSP's and IPSP's, respectively.

These data provide evidence that repeated presentations of a glabella tap over a period of two days increases the proportion of cells within the motor cortex responding to the stimulus with increased activity. Furthermore, ECS US which reinforces eyelink conditioning produces EPSP's and IPSP's in the motor cortex. (Supp. by ANSOR 76-3074 and BNS 78-24146.)


Previous work shows that Spirostomum ambiguum responds to mechanical (vibratory) and electrical stimulation by rapidly contracting. Repeated mechanical but not electrical stimulation (rate 0.1 Hz) leads to a decrement in the probability (habituation) of the contractile response. The independence of the two stimulation modes as to habituation and contractile sites for mecano- and electro-transduction (Osborn et al., Behav. Biol. 8: 655, 1972). Earlier studies also indicated that the extracellular calcium is important in mechanotransduction (Osborn et al., Behav. Biol. 8: 655, 1972).

The present study is concerned with the effects of extracellular calcium on the transduction and habituation processes in Spirostomum. Calcium was manipulated by incubating the protozoans in media containing different Calcium concentrations and by the addition of the calcium chelator ethylenebisdiamine-tetraacetic acid (EDTA). The results indicate that increasing the calcium concentration increases responsiveness to mechanical stimulation over the concentration range studied (0-0.5mM). Initial responsiveness (minute 1 of the stimulation period) to electrical stimulation was depressed in media to which no calcium was added. Such media also result in the lack of extracellular calcium causes a decrement in responsiveness to mechanical (vibratory) and electrical stimulation by rapidly contracting. Repeated mechanical but not electrical stimulation (rate 0.1 Hz) leads to a decrement in the probability (habituation) of the contractile response. The independence of the two stimulation modes as to habituation and contractile sites for mecano- and electro-transduction (Osborn et al., Behav. Biol. 8: 655, 1972).

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1034 EFFECTS OF L-PROLINE, PROLINE ANALOGS AND OTHER AMINO ACIDS ON MEMORY IN THE CHICK.

Arthur Cherkin and Joel L. Davis. GREEC and Psychobiology Research Laboratory, VA Medical Center, Sepulveda, CA 91343.

The amnestic effect of L-proline (L-PRO) (Van Harreveld and Fifiokova, Brain Res. 1970, 85, 165) has been confirmed by two laboratories (Science 193, 242, 1976; Neurosci. Lett. 6, 355, 1977). The latter concludes that the amnestic effect results from interference with brain amino acid uptake rather than a glutamate release as postulated by Van Harreveld and Fifiokova. Structure-function relationships may clarify the issue. The L-PRO effect is stereospecific and dependent upon the molecule (Brain Res. 155, 256, 1978). We have reported the amnestic effect of DL-3,4-dehydroproline and L-baltsikalin. We now present data on additional amino acids. We injected chicks intracerebrally with 10 µl/hemisphere of 300 mM amino acid, 1 min after one-trial training to suppress the peck response to a bead. Suppression was constituted by coating the bead with an aqueous solution of an amino acid containing 10 µg/mg. Amino acids were selected from an extensive survey of 99 amino acids. The experiment was in block design. The results with D-proline and cyclocelulose are similar to those with no injection, eliminating trivial factors, such as injection itself, as causative of memory impairment. The results with L-isoleucine, and with L-serine and L-threonine (albeit borderline), indicate that imbalance of free amino acids in the ventricular fluid does not necessarily impair memory. Of interest is that the amino acids found by Van Harreveld (Neuroscience Abst., this volume) to resemble L-PRO in their dose-response effect upon spreading depression in the chick retina (L-serine, L-proline, L-threonine, L-baltsikalin, 4-hydroxy-L-proline) also have an amnestic effect. The amnestic effect of amino acids which do not resemble the retina experiment (glycine, L-homoserine, L-glutamine) may result from different mechanisms.


Performance of amnesic patients on tests about public events or public figures has previously been evaluated, usually in a single population of patients. The present study assessed memory for photographs of famous individuals who came into prominence at various times from the 1930s through the 1970s. We studied the amnesic patient N.A., a group of alcoholic Korsakoff patients, and three groups of control subjects.

Three major findings emerged: 1) the pattern of impairment demonstrated by N.A. differed markedly from that demonstrated by the Korsakoffs. N.A. scored at least as well as matched controls on photographs of individuals who were known prior to the onset of his amnesia in 1960. N.A. was severely impaired with photographs of individuals who were known only in the 1960s or 1970s. In contrast, Korsakoff patients were impaired over the entire range of the present study and their performance was poorer for the recent time periods than for remote periods. 2) Yes/no and multiple-choice cuing improved both amnesic performance and control performance. The findings provide support for the notion that the marked temporal gradient of impairment demonstrated by the Korsakoffs might in part reflect the gradual onset of anterograde amnesia over many years. Taken together, these findings emphasize the difference between etiologically distinct amnesias and provide additional evidence for continuity between Korsakoff patients and chronic alcoholics.
A MODEL FOR PERCEPTUAL CODING BASED ON EEG MEDIANDED BY NOVEL COLOR AND TASTE CUES, RESPECTIVELY.

Half of the brain can independently acquire an illness-induced food aversion, with learning in the seeing and non-seeing hemispheres to the colored sucrose, but not to uncolored sucrose, regardless of deprivation during training. Chicks tested with the trained eye displayed a marked aversion to LiCl after drinking green sucrose solution. Half the animals adapted to the familiar sucrose taste. Chicks with one eye open for learned aversions to green or uncolored sucrose. Most candidates for neurobiological correlates of retrograde amnesia (e.g., seizures, or transmitter or protein synthesis inhibition) have only limited generalization across amnestic agents. Recently we found that brain norepinephrine (NE) concentrations are sensitive to several training-treatment combinations. The extent of a transient decrease (maximal 10 min after training) in NE content appears to be a predictor of later retention performance. Rats received footshock for retention performance 24 hr later; both cortical stimulation and lesions confined to the central nucleus of the amygdala produce impairment of conditioned bradycardia in rabbits (Kapp et al., Neurosci. Abs., 1978). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation to heart rate conditioning demonstrated that lesions confined to the central nucleus of the amygdala produce impairment of conditioned heart rate conditional bradycardia in rabbits (Kapp et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532).
1042 HOW DOES A BRAIN BUILD A COGNITIVE CODE? Stephen Grossberg.  

This talk addresses the question: where codes of sensory or cognitive events can develop through experience, how are globally consistent hierarchies of such codes established and maintained? How is the local ignorance of each feature detector reconciled to yield global consensus? How is a coding error corrected when no individual cell knows that one has occurred? Previous work has shown that feedback coding mechanisms are either unstable in a complex input environment, or must be shut off by a chemical switch that is insensitive to the behavioral meaning of the code. This paper indicates how competition between different data learned and learned expectations, or templates, can stabilize the code by buffering committed populations against continual erosion by new environmental demands. The gating phenomena that result lead to dynamically maintained critical periods, whether or not a chemical switch also exists, and to attentional phenomena such as overshadowing in the adult. The functional unit of cognitive coding is suggested to be an adaptive resonance, or amplification and prolongation of neural activity, that occurs when afferent data and effector expectancies reach consensus through a matching process. The resonant state is a global, context-dependent representation of the data in STM. In STM, mismatch between afferent data and efferent expectancies are capable of driving slow LTM changes in the codes and expectancies that define the network. Mismatch between afferent data and effector expectancies yields a global suppression of activity, and triggers a reset of codes in STM, as well as a rapid parallel search for unmatched codes. This is accomplished by a specific arousal mechanism that is gated by a chemical transmitter system, probably catecholaminergic, whose relative states of accumulation at antagonistic pairs, or dyads, can change the pattern of STM activity across a field of feature detectors. In particular, a sudden arousal increment in response to an unperceived input, or mismatch, can selectively reverse, or rebound, these relative activities in a graded fashion across all cells, thereby suppressing incorrectly classified populations. The cellular field is harnessed to a rapid parallel scheme of hypothesis testing is elicited. A source paper is in Progress in Theoret. Biol., Vol. 5. R. Rosen and F. Snell, Eds. New York: Academic Press 1978.


Mice were reared in either enriched, social control or isolated environments for 30 days. Environmental enrichment results in a significant and selective increase of paradoxical sleep (PS, or REM sleep) and also enhances recall of a multiple-trial, in the dark discrimination task after training. Conversely, isolated mice exhibit a decrease in PS and impaired task performance relative to controls. These results support the hypothesis that PS plays an integral role in the maintenance and stability of long-term memory.

<table>
<thead>
<tr>
<th>Acquisition</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE</td>
<td>41.84±2.28</td>
<td>45.74±2.51</td>
<td>48.62±3.35</td>
</tr>
<tr>
<td>(N=38) a</td>
<td>p&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>40.64±3.31</td>
<td>48.63±3.31</td>
<td>54.34±3.20</td>
</tr>
<tr>
<td>(N=19) a</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TE</td>
<td>36.04±2.41</td>
<td>41.52±2.72</td>
<td>53.12±2.79</td>
</tr>
<tr>
<td>(N=18) a</td>
<td>p&lt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1044 POSTNATAL MALNUTRITION AND ITS EFFECTS ON FOOD MOTIVATION OF ADULT RATS: IMPLICATIONS FOR LEARNING STUDIES. Edward S. Halas, Patricia A. Burger*, and Harold H. Sandstead*.
Dept. Psychology, University of North Dakota and Human Nutrition Lab., USDA, Grand Forks, N. D. 58202

Several studies have shown that prenatal and postnatal malnutrition impaired subsequent performance of animals on learning tasks that involved food motivation. These experiments are difficult to interpret because the food motivation of animals which have been deprived of food may not be the same as normal animals. Two different types of postnatal malnutrition were studied. Ten pregnant Long-Evans rats were given a zinc deficient diet (ZD) and separated from their mothers. The control dams were given 25 ppm/Zinc in their water. A third group of 10 dams (AL) were fed the diet ad libitum plus the zinc supplemented water. After weaning, all pups were fed Chow ad libitum plus tap water. At 90 days of age, the offspring began their food motivation test. Six males and 6 females were selected from each of the 3 dietary groups for a total of 36 rats. Once a week for the next 8 weeks, each pup was deprived of food for 24 hours and tested for food motivation in an operant box using a progressive ratio schedule. The ratio was increased by one after every reward. The "breaking point" was defined as failure to bar press for 15 min. The PF rats, both males and females, had significantly higher breaking points than the AL males and females. The higher food motivation of the postnataally malnourished rats (ZD and PF) has definite implications for learning studies. Performance on a learning task is affected by two critical variables: learning ability and motivation. When learning is studied, the motivation of the animals must be constant, otherwise a difference in performance between groups cannot be attributed to a difference in learning ability. These difficulties of interpretation are further complicated when the Yerkes-Dobson law is taken into consideration.

This work was supported in part by the USDA Cooperative Agreement 1S1-14-3001-294 with the University of North Dakota.

1045 THE ROLE OF THE HIPPOCAMPAL FORMATION IN HUMAN MEMORY: A MODEL. Eric Haigren, Brain Research Institute, UCLA, Los Angeles, CA 90024.

The preservation of general intellectual processes with the narrow exception of Recent Memory (RecM) after bilateral Hipocampal Formation (HCF) damage implies that these processes are localized elsewhere: the model assumes that they are performed by the neocortex (NC) and that the brain processes correlated directly with the specific contents of a conscious experience lies in a cell-assembly(CA) distributed throughout widespread NC areas. Residual excitation intrinsic to just-activated NCCAs underlies Short Term Memory. Remote Memory (RemM), also intact after HCF lesions, proceeds using cognitive processes such as logical deduction, acting on general knowledge of the world and specific knowledge of the item to be recalled. When a NCCA is activated, a portion of its efferents converge on a group of Hippocampal (HCF) neurons. Very powerful and long-lasting post-tetanotic-potentiation is known to occur within the HCF. As a result of this potentiation, a NCCA activated by a certain set of afferents will fire when a subset of these afferents are later activated, i.e., when a semantic cue is re-experienced. Persistent or repeated activation of a NCCA will evoke progressively stronger and specific activation of the associated HCF neurons in the same lamina. There are several ways whereby the activation of an HCFCA by a cue could promote the activation of the NCCA associated with this afferent: (1) direct projections of HCF neurons to NC, several of which have described, although they have not been demonstrated to be topographically reciprocal; (2) direct antihormonal activation of a NCCA synapse upon the HFCNCA, perhaps only when the HCFCA is sufficiently excited by positive feedback via the HCF. (3) direct and indirect subicular cortex (HCF) efferents to the nonspecific thalamic nuclei may help focus attention on relevant NC areas, in concert with the aforementioned cognitive processes involved in both RecM and RemM, lead to the activation of further cues. A cyclical process would thus be initiated that, if successful, would culminate in an exploration of the reactivated potential of the afferent cue as experience and as a feeling of recognition. Our model is consistent with existing data, integrates with neural and cognitive models elaborated at different levels of analysis, suggests experiments, and makes testable predictions. (Supported by grants NSF-BNS77-17070 and NIH-NI02808. My thanks to Thomas Babb and Paul Crandall)

The effect of unilateral carotid ligation on striatal catecholamine fluorescence (CAF), learning and memory was studied in the gerbil. Animals were anesthetized with ether, the left carotid artery exposed and ligated, and surgery was performed at 5 and 15 minutes, 24 and 48 hours and up to 3 weeks post-ligation. The left and right caudate (LC, RC) were examined for CAF by the fast-kinetic fluorescence technique. Learning and memory were studied with a shuttle box by conditioned avoidance response (CAR) acquisition and retention. Animals were observed for 24 hours to 17 days post-ligation for behavioral changes. Although no alterations in performance were observed in the LC than RC after 24 hours, and a further drop by 48 hours. By 3 weeks, there was a return of CAF in the LC and RC. Acquisition of the CAR was delayed by 20% (from 97 CAR/animal to 77 CAR/animal) in animals tested 48 hours post-ligation, and by 28% (from 71 CAR/animal to 51 CAR/animal) when tested 15 days after ligation. Memory seemed more affected by ligation; animals tested at different times between 2 hours and 10 days after surgery showed no decrease in CAR’s. The data indicate that bilateral changes occur in caudate CAF of both hemispheres after unilateral carotid ligation which are apparently not correlated with either learning nor memory behavior.

SPLIT-BRAIN HUMANS. Larry E. Johnson* (SPON: James Bonner), Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Split-brain humans can verbally categorize left visual field (LVF) stimuli and compare stimuli presented simultaneously in the LVF and RVF. This contrasts with the theory of 'complete disconnection' in which communication between the two cerebral hemispheres following commissurotomy is severely limited. All the split-brain subjects tested (4), when required to distinguish tachistoscopically presented letters and numbers from patterns, or identify single digit numbers as greater or less than '5', or odd-even (choice discriminations), can do so very accurately in both visual fields using either manual or verbal responses. And, like normal or partially split controls, they also respond as fast to stimuli in one field as the other. When asked to name stimuli, however, LVF accuracy varies between sub­jects and differs for various stimuli and sample sizes. Yet, in spite of these individual differences and in contrast with normals and a partial split, all of the split-brain subjects are alike in that they require significantly longer to name, whether correctly or incorrectly, LVF as compared with RVF stimuli. When stimuli (colors, patterns, numbers, or letters) are presented simultaneously to the two visual fields, the ability to manually or verbally categorize two stimuli as same or different seems to be dissociated from the ability to name the stimuli.

These findings demonstrate that cholinergic and noradrenergic agents can modulate the disruptive effect that cycloheximide has on learning. With respect to the aspect of learning and memory that is impaired, it is clear that cycloheximide did not affect acquisition of the initial problem but did affect the acquisition and retention of the reversal problem. These results suggest that cycloheximide either induced perseveration of the initial behavior or enhanced proactive interference. Therefore, cycloheximide may influence both learning and memory. [Supported by NIMH Grant MH 11167 and NIGMS Grant GM 07305.]


Previous work has shown that bilateral injection of 10 or 20 µg of cycloheximide (CIH) into the amygdala, but not into the internal capsule, resulted in a time-dependent disruption of long-term retention of passive avoidance training, even though total brain protein synthesis was inhibited by less than 6%. In the present study, autoradiograms were made following injection with L-[methyl-14C]-methionine following intracranial administration of CIH via implanted cannulas in either the amygdala or internal capsule. Autoradiograms from different brain levels were analyzed by use of an image-analyzing computer to quantitate the regional inhibition of protein synthesis. Control experiments showed that the image analyzer produced accurate optical density measurements and that these values were linearly related to the amount of radioactivity. Data indicate that the results obtained with this new histoanalytic technique do not differ from traditional biochemical techniques and the degree of inhibition of protein synthesis within the amygdala, internal capsule, caudate, cortex, hippocampus, thalamus, hypothalamus, and half brain following 20 µg CIH injection into the amygdala is both replicable and internally consistent. Furthermore, a dose-dependent relationship exists between degree of inhibition and injection site. In addition, following amygdaloid injections of 20 µg CIH, a profound inhibition of protein synthesis (-60%) is found within the amygdala and internal capsule, but similar injections given with cycloheximide result in marked inhibition in the internal capsule, but very little, if any, inhibition in the subjacent amygdala. In general, this new histoanalytic technique provides for a new quantitative approach for analyzing autoradiograms. The technique appears to be accurate, reliable and can be applied to the study of behavioral functions. Indeed, it is suggested that based on autoradiographic and behavioral data, CIH injection of CIH impairs memory by virtue of its action upon amygdala function.
1050 FACILITATION OF EYE-BLINK CONDITIONING BY HYPOTHALAMIC STIMULATION. H.-J. Kim and C.D. Hoody. UCLA Medical Center, Los Angeles CA. 90024.

"More than 1000 pairings of click (CS) and glabella tap (US) may be needed to attain stable, short-latency conditioned eye blink responses in the cat (Noody et al. J. Neurophysiol. 1974). Some time ago Murphy and Fellhorn (J. Neurophysiol. 1965) found that hypothalamic stimulation (HS) could facilitate the acquisition of electrocorticographically elicited movements in an "enduring" manner. Voronin (Proc. Intl. Union Physiol. Sci. 1974) reported that adding HS to the auditory CS US consisting of electric stimulation of the motor cortex resulted in rapid acquisition of a short-latency conditioned startle response. He has suggested that the effect is associative.

We report the following preliminary results of pairing click CS with glabella tap (ISI, 340 ms) and HS (240 ms after tap). A high of intensity comparable to click CS was also presented 4 sec after HS as a discriminative stimulus (DS) and CRs were extinguished within 30-50 pairings or less.

1. CRs emerged within 30-50 pairings or less.
2. The onset latencies of the major blink responses to HS, measured electroencephalographically, ranged between 80 and 320 msec.
3. CRs were extinguished when click and HS were presented alone.
4. Responses elicited by the ISI were smaller and less frequently observed than those elicited by the CS. Initially, before any associative pairing, subliminal myographic activity was greater to HS than to CS.
5. CRs were also smaller and less frequently observed when delivery of HS was moved to before click CS, tap, and HS ISI.
6. The effect of HS was location-dependent within the hypothalamus and was not seen with stimulation of the adjacent optic chiasma, which produced pronounced blinking.
7. Other effects that were also produced including somatic-sensitization, a facilitation of the response to glabella tap, and small, short-latency (onset: 15-50 msec) responses to click and frequently to HS.

Intracerebral recordings obtained from awake cats during these procedures indicate that the major effects of HS on the activity of cells in the motor cortex are: a) early excitation, b) late inhibition, and c) later excitation. (Supp. by AFOSR 76-3074 and NSF BNS 78-24146.)


Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

There is considerable evidence that retention of learned responses is impaired by posttrial electrical stimulation of the amygdala. These findings suggest that the amygdala may be involved in modulating memory consolidation. However, the possibility is that the amygdala is in some way involved in the persistent storage of memory. If the amygdala is involved primarily in modulating memory consolidation, then the impairing effect of a lesion given after training should increase as the time before the training and the retention test is increased. To investigate this issue, bipolar electrodes were implanted bilaterally into the amygdaloid of male RAS Sprague-Dawley rats (60 days old). Following surgery, the animals were trained on an inhibitory avoidance task using a 2 mA, 2 sec footshock, and tested after one of three retention intervals: 4 days, 7 days, 12 days. Five groups tested at each retention interval: unimplanted control (UC), implanted control (IC), pre-training lesion (IL), and delayed posttraining lesion (DL) (2 days before training). The time between the training experience and delayed posttraining test was 2 days, 5 days, or 10 days for the various training retention intervals used. Control animals from these 5 groups were trained without footshock and tested at the 12 day retention interval. Bilateral amygdaloid lesions were made with radio frequency current (1,5-2,0 mA, 30 sec). Histological results showed the lesion area included primarily the lateral, basolateral, and basomedial nuclei as well as part of the pyriform cortex. As is shown in the table, a retention deficit occurred only when the amygdaloid lesion was made at least 1 day following training. This time-dependency supports the notion that the amygdala is involved in memory modulation rather than as a site of memory storage.

<table>
<thead>
<tr>
<th>Retention Interval (sec)</th>
<th>UC</th>
<th>IC</th>
<th>PL</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>600</td>
<td>417.4</td>
<td>56.9</td>
<td>58.3</td>
</tr>
<tr>
<td>7 days</td>
<td>600</td>
<td>185.9</td>
<td>14.9</td>
<td>24.8</td>
</tr>
<tr>
<td>12 days</td>
<td>600</td>
<td>100.5</td>
<td>11.7</td>
<td>12.8</td>
</tr>
<tr>
<td>24 days</td>
<td>600</td>
<td>100.5</td>
<td>11.7</td>
<td>12.8</td>
</tr>
</tbody>
</table>

*p < .05 different from IC; **p < .05 different from IC

Supported by UPHS grants MH 12529, AG 00538; BNS 76-17370 and a grant from the McNICHOL Foundation (all to JMLG).


Pharmacology Department, Warner-Lambert/Parke-Davis Research Laboratories, Ann Arbor, MI 48105.

Benzodiazepines have been found to impair short-term memory (STM) in humans. The effects of diazepam on a non-human primate model of STM were examined to explore further the amnestic effects reported in humans. Young and aged animals were used to test for possible age differences in response to diazepam treatment.

In Experiment I, five young rhesus monkeys (5-8 years, 4.5-5.1 kg) were tested on an indirect delayed-response task. The Automated General Experimental Device (AGED) was used to measure the animals' ability to recall the spatial position of a stimulus presented prior to retarding an interval of various durations. Two retention conditions were used: a 0-sec, non-memory control condition; and a 60 sec, STM-dependent condition. Diazepam, at doses of 2.5, 5.0, and 10.0 mg/kg administered orally 30 min prior to behavioral testing, produced a dose-related decrease in delayed response accuracy only at the 60 sec, memory-dependent retention interval (drug x condition interaction, p<.05). However, no improvement was found on the 0-sec control condition, indicating that memory per se was affected by the drug and not non-specific, performance aspects of the task.

In Experiment II, six aged rhesus monkeys (18+ years, 4.2-5.4 kg) were tested on the same task using the same apparatus as in Experiment I. However, the retention conditions were used: a 0-sec, control condition; a 'short' delay, adjusted for each individual animal to yield a control performance level of 60-80% correct (range = 47-62, SEM = 5); and a 'long' delay, yielding 40-60% correct performance level (range = 33-69). Diazepam (1.25 and 2.5 mg/kg administered orally 30 min prior to testing produced declines in performance levels in the 0-sec control conditions (p<.01). These effects are similar to those found in young animals, although more severe. Again, the amount of memory impairment was dose-dependent within the range of doses that produced a greater disruption of delayed-response performance.

Thus, the short-term memory impairments produced by diazepam in these studies are not dependent on age and are similar to those reported in humans. They also correspond to the well-known impairments of STM produced by the administration of anti-cholinergic drugs to animals and humans. Diazepam is reported to block the release of acetylcholine in the brain, the amnestic effects of diazepam may be due to its effects on the cholinergic system. Further, these findings may provide a useful model of memory impairments commonly associated with human brain disorders.

Supported by McNichol Foundation, NINCDS Fellowship 5 F32 NS05694-02, NSF (BMS-75-00453).
1054 ADRENAi MEMORY AND LEARNING

1056 PLASMA CATEChOLAMINES RESPONSES TO TRAINING AND POSTTRIAL MEMORY

1065 MEMORY, TESTABILITY & AFFECT IN LATE-ONSET DEMENTIA. Miller, N.E., Beatriz J. Vasquez, Robert A. Jensen, Rita B. Messing, Henk Rijnt*. K.C. Littg, and James J. McLaugh. Department of Psychology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

In the first experiment, male Sprague-Dawley rats were trained in a one-trial inhibitory avoidance task (3 mA, 2 sec FS). Immediately following train ing, they were exposed to several tests designed to evaluate 1. Long term memory for the shock, 2. short term memory for the shock, and 3. extinction of the learned response. Animals were trained using a drug with limited ability to cross the blood brain barrier, on plasma samples (0.5 ml) were obtained from previously implanted tail artery catheters immediately and 5, 10, 20, and 40 min after the various treatments. Plasma levels of EPI and NE were determined using a COI of radiometric-thin layer chromatographic procedure.

The results indicated that FS or FS + FCX resulted in an approximately 10-fold increase in plasma EPI immediately after the treatment. FCX alone did not result in an increase in plasma EPI concentration above that of non-footshocked or non-stimulated animals. The concentrations of EPI for all groups returned to basal values within 40 minutes. A similar pattern, but of lesser magnitude, was observed for plasma levels of NE.

In a second experiment (without training), posttraining injections of ACTH (0.3, 1.0 I.U./rat) or ACTH analog (Organon 2766: 125 or 250 mg/kg) failed to alter either plasma EPI or NE concentrations. An EPI injection (i.e.) at a dose of 250 mg/kg that decreased plasma EP1 concentrations in an increase within 5 min in plasma EPI concentrations from 230 pg/ml to approximately 1000 pg/ml that was maintained for 40 min. A higher dose of ACTH that caused a rise in plasma NE concentrations and amnesia resulted in plasma EPI concentrations of approximately 4000 pg/ml during the 50 min after injection. Plasma NE concentrations did not change following the injection.

These results indicate that peripheral adrenergic responses do occur with training. Furthermore, an injection of EPI at a dose that enhances performance may mimic a component of the endogenous responses to a training FS. However, other treatments that modulate retention (i.e., FCX or ACTH) may not involve sympathoadrenal medullary activity.

Supported by U.S.P.H.S. research grant MH 3141.

1066 MEMORY, TESTABILITY & AFFECT IN LATE-ONSET DEMENTIA. Miller, N.E. et al. for Studies of the MB of the Aging. NIMH, Rockville, Md.

Behavioral measures of memory are important tools in the assessment of treatment efficacy in senile dementia. Yet clinical researchers often report that aged patients with altered brain function are untreatable on both standard and newer experimental tests of memory. As part of a continuing investigation of memory impairment in late life psychiatric disorders, a series of experiments was performed to study the behavioral characteristics of memory positive for diffuse, chronic, organic brain disease. 11 2S with altered brain function and 17 controls aged 50 and up were tested on a choice reaction time test derived from Sternberg. Digit strings ranging in length from 1 to 6 digits were displayed in a sequential format for 3 seconds, followed by a 2 second delay. After a 2 second delay and a warning buzzer, the digit was displayed. The task was to decide whether the digit had appeared in the preceding sequence. Despite the presence of a 2 second delay and a 2 second delay and a warning buzzer, the digit was displayed. The task was to decide whether the digit had appeared in the preceding sequence.

In both experiments, rhesus monkeys were trained preoperatively on a one-trial visual recognition task requiring memory of single objects for 10 seconds. Two new experiments show that the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems.

In the first experiment, bilateral amygdala-hippocampus in monkeys yields a memory impairment resembling the clinical syndrome of global anterograde amnesia (Mishkin, Nature 273: 297, 1978; Spiegel & Mishkin, Learn. Mem. 5; 1979). Two new experiments show that the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems.

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1060 EVIDENCE THAT HIRANO BODIES IN HUMAN BRAIN ARE PARACRYSTALLINE
memory associated proteins. Microscopy shows that the Hirano body binds acridine orange and were subsequently analyzed by sequential Fourier transform age and the ribosomes are not available for the synthesis of positive results were obtained when the bodies were stained for acid mucoprotein. Rat liver and shows its distinctive asymmetric "skiff" shape. These particles have a highly polar distribution within the electron dense particle was produced. This image is virtually identical images in electron micrographs to digitized data arrays which positive similarity to membrane-bound ribosomes and we believe staining characteristics and other morphological features suggest that these data further support our working hypothesis. The Hirano bodies represent a crystalline form of rough endoplasmic reticulum. An Optronics high speed microdensitometer results were obtained that show the bodies to be composed of sheets of electron dense particulate matter. An Optronics high speed microdensitometer interfaced to a PDP11/40 computer was used to convert periodic images in electron micrographs to digitized data arrays which were subsequently analyzed by sequential Fourier transform methods. A digitally filtered and averaged image of the electron dense particle was produced. This image is virtually identical to that obtained for the 60s subunit of ribosomal particle from rat liver and shows its distinctive asymmetric "skiff" shape. Histochemical analysis of formalin fixed, paraffin embedded autopsy samples included specific staining for nucleic acids. Positive results were obtained with toluidine blue, ethidium bromide, and acridine orange. Concomitant light and fluorescence microscopy shows that the Hirano body binds acridine orange and fluoresces with a red component as expected for RNA; nuclei fluoresce yellow-green of DNA. Mitochondrial material in adjacent cells also fluoresces red. Negative results were obtained when the bodies were stained for acid mucoprotein, carbohydrates with periodic acid-Schiff. In red and blue light, electron microscopy shows that Hirano bodies are ordered, crystalline arrays of membrane-bound ribosomes. The hippocampal region is associated with the consolidation of short term to long term memory and the predominant symptom of senile dementia is the impaired capacity to consolidate new memory. Thus the possibility arises that these bodies represent a form of ribosomal storage and the ribosomes are not available for the synthesis of memory associated proteins.

The role of the LC in complex behavior remains elusive. Recent studies from our laboratory reported that unilateral, but not bilateral, post-training LC lesions resulted in a dramatic (70%) extension of the susceptibility period to ECS-produced retrograde (RA) amnesia. The present study extends the analyses of the role of the LC in memory by investigating the effect of pre-training LC lesions on the consequences of ECS upon memory.

Male Swiss mice (n=291) received small electrolytic lesions (500 uA anodal current for 10 sec.) delivered through twisted nichrome wire electrodes (125 uA). Either 2 or 14 days after lesioning, mice were trained in a single trial inhibitory avoidance task (500 uA) and immediately returned to their home cages. After either a 24 hr or 14 day post-training period, transcorneal ECS or sham ECS (15.0 mA for 200 ms) was administered. All mice were tested for retention of the inhibitory avoidance response 24 hrs after ECS.

The results indicate that in all cases unilateral LC damage resulted in an extended susceptibility to ECS. These results are compatible with, and extend, previous data from our laboratory using post-training lesions.

The most interesting result from this experiment is that ECS resulted, paradoxically, in facilitation of performance (p = .04) in mice receiving bilateral, but not unilateral, LC damage 28 days prior to ECS. Bilaterally, but not unilaterally lesioned animals not receiving ECS, had accelerated forgetting when compared to control lesioned mice (p = .01). This facilitated performance in bilaterally lesioned mice receiving ECS appears to result from the stimulation of the residual memory remaining after 14 days of accelerated forgetting in similarly-lesioned mice not given ECS. Control lesioned mice, either with or without ECS, displayed the no recall response. It will be discussed in terms of the possible dynamic changes occurring in neurotransmitter systems mediating memory storage processes, and the role of the LC and of ECS in altering these dynamic changes.

(This research supported by a Sloan Research Fellowship to S. F. 2.)


It is commonly observed that it takes longer to recall verbal information as a person grows older. There is a reduction in the number of baseline trials which can be related more directly to the rate of scanning of items in memory, to the verbal memory load, or to the decision making (Pozard & Poon, 1978). We hypothesized that hormonal influences on memory or incorrectness of a recognition response influence decision times and that these would be age dependent.

Young, Middle-Aged, and Old pigeons (n = 57) were asked to answer as quickly as possible during recognition of 10 visual nonverbal designs recurring randomly in a series of 40. From unweighted means and signal detection results we found that memory load, or to the decision making (Fozard & Poon, 1978). We hypothesized that the perceived correctness or incorrectness of a recognition response influence decision times and that these would be age dependent.

Young, Middle-Aged, and Old pigeons (n = 57) were asked to answer as quickly as possible during recognition of 10 visual nonverbal designs recurring randomly in a series of 40. From unweighted means and signal detection analyses we observed that age groups differed in the decision criteria adopted for recurrent recognition (P = 0.05). Regardless of age, however, the response times differed significantly among correct, false, missed and rejected recognitions (P < 0.001). More specifically, the average time for correct recognitions was smaller (P < 0.01) than that for false or for missed recognitions. The results argue for a response delay of approximately 80 msec. Independent items fail to meet the parity check for recognition.

1065 AMYGDALOID MULTIPLE UNIT ACTIVITY DURING CLASSICAL CONDITIONING IN RABBITS. Russell T. Richardson* and Richard F. Thompson, Dept. of Psychology, Univ. of Guelph, Irvine, CA 92717.

Experimental manipulations of the amygdala can alter learning processes. For example, Kapp et al. (Neurosci. Abst. 3:236, 1977) have shown that lesions of the amygdala can retard conditioning of the nictitating membrane reflex in rabbits. However, to further investigate the role of the amygdala in learning, we recorded multiple unit activity bilaterally from the amygdalae of New Zealand white rabbits during classical conditioning of the nictitating membrane reflex. The training consisted of a 1 kHz tone (CS) presented 250 msec prior to a 1000 msec puff of air to the conjunctiva (US). The effects of lesioning and sham operations on the multiple unit activity in the CS and US periods was examined using two different pretraining presentations. The second group received only two days of paired training. Multiple unit activity was recorded from CS onset (PreCS period), 250 msec after CS onset (CS period), and 250 msec after US onset (US period). All CS and US period count rates were expressed as percent increases over the preceding PreCS period count.

The multiple unit activity during paired stimulus presentations showed a strong increase (121%) in the US period. However, this increase was not significantly different from that seen during unpaired stimulus presentations, and it did not show consistent changes as animals acquired the conditioned response. There was an average increase of 34% in activity during the CS period on paired training days but, again, this response was not significantly different from the response on unpaired training days. Several cell populations showed a significant increase in firing in the CS period on both paired training days, and both of these populations were found in or adjacent to the cortical nucleus of the amygdala. Activity during the PreCS period showed a highly significant (p<.001) increase in firing over the training session on both paired and unpaired days. The CS and US period count rates also increased during the training days, but because they were assessed relative to the PreCS counting, their activity is shown changing each day.

These findings indicate that, in contrast to the hippocampus, the amygdala multiple unit activity in the amygdala does not display changes which can be strongly correlated with acquisition of the conditioned response.

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Facilitation of a visually discriminated avoidance learning was observed in rats one week after they were lesioned in the dorsal hippocampus. While perseveration of a spatial strategy acquired during a pre-training session occurred in sham-operated behaviorally lesioned controls, no such perseveration was observed in hippocampal lesions. Elimination of the pre-surgical training session reduced the number of spatial strategies in all groups. Significant differences in trials to criterion and percentage of place strategies remained. Testing animals 3 weeks after training showed that hippocampal and cortical differences had poorer retention than sham, while there were no differences in retention groups tested 24 h after training.

If the lesion is made weeks prior to training, the same effect is seen on rate of acquisition and on perseveration of spatial strategies as when the lesion precedes the training by one week. Thus the behavioral differences seen after the 3 week training to test interval cannot be due to a recovery of hippocampal function.

A further study examined the effect of the lesion on long term retention of the discrimination acquired before surgery. It was found that there was no retention deficit under these conditions. The similarity between these results and the clinical hippocampal syndrome suggests that this preparation might be useful as an animal model for pharmacological studies aimed at alleviation of amnesia.

The nature of the memory deficit (e.g., increased rate of decay or retrieval dysfunction) remains to be investigated. Studies are presently underway to determine what conditions this deficit can be reversed by increasing retrieval cues at retention testing or by using a reactivation procedure during the training to test interval.


Adult female Wistar rats were submitted to the following behavioral treatments in a shuttle-box: 1. pseudoconditioning (5-sec tones and 1 mA footshocks at random); 2. Pavlovian conditioning (tone-shock pairings on every trial regardless of responses made to the former); 3. avoidance without CS-US pairing (each tone followed at a randomly varying interval of 35 sec unless there was a shuttle response to the tone); 4. tones alone; 5. shocks alone. Intertrial interval was 10-40 SEC in all cases. Sessions were 5 or 25 min long. Two biochemical parameters were measured in hippocampus, caudate nucleus, rest-of-the-brain, and liver of these animals: a) in vivo incorporation of 32-P to non-histone acid-extractable chromosomal proteins (NAEP); b) in vitro incorporation of 14-C-leucine to total protein. Data were expressed as dpm/mg NAEP / dpm/mg total protein in the 32-P experiments, and as dpm/mg protein / dpm in total homogenate in the 14-C experiments. Statistics were by multiple-range analysis (Null hypothesis rejected at 1 or 5% level).

There was an increased phosphorylation of hippocampal and caudate NAEPs after 5 min of Pavlovian or avoidance learning, and of the rest-of-brain fraction after 25 min of shocks alone. No change relative to intact controls was detected in the other groups. An increased incorporation of 14-C-leucine into hippocampal and caudate total proteins was observed after 25 min of avoidance training, 1 hr later in animals submitted to either tones or shocks alone, and 2 hr later in rats submitted to the Pavlovian procedure. These results argue against a specificity of the biochemical parameters examined to learning or memory processes; but point out, instead, to a selective effect of the learning factors studied on their time course to a positive influence of pseudoconditioning on their occurrence. The latter is of importance since pseudoconditioning has repeatedly been shown to be an inseparable component of aversive learning, classical or instrumental.


Damage limited to the hippocampus of monkeys has failed to reproduce the profound anterograde amnesia that has been attributed to such damage in man. By contrast, combined damage to the monkey's hippocampus and amygdala does appear to reproduce the syndrome. A severe memory loss following combined amygdalo-hippocampal ablation was found recently on a visual recognition task in which the animal had to decide on the basis of a single previous experience whether a test object was familiar or novel (i.e., had previously been presented or not) (Mishkin, Nature 273: 297, 1978). The present study demonstrates that the impairment following the combined limbic lesion is not limited to such stimulus recognition memory, but extends to stimulus-reward associative memory as well.

The rhesus monkeys that served as subjects were part of an earlier study of associative memory (Spiegler & Mishkin, Neurosci. Abstr. 4: 263, 1978) in which the animals had to decide on the basis of a single previous experience whether a test object was positive or negative (i.e., had previously been baited or not). It was found in that study that bilateral lesions of either area or to the temporal limbic area. Thus, animals originally given TE lesions were now given adjacent TEO lesions, and vice versa; similarly, lesions originally given amygdala lesions now received adjacent hippocampal lesions, and vice versa.

The effect of an additional cortical lesion was indistinguishable from the effects of that particular lesion given by itself. That is, the addition of a TE to a TEO removal produced the same marked-but-recoverable deficit as a TE removal alone, while the addition of a TEO to a TE removal produced the same negligible effect as a TEO removal alone. Only when a bilateral prestriate ablation was added in a third stage did a severe and lasting deficit ensue. By contrast, the serial limbic lesions produced a seemingly permanent loss irrespective of the sequence of lesions. None of these animals reestablished one-trial learning of object-reward associations within three sessions of postoperative testing. Apparently, the amygdala and hippocampus can serve as partial substitutes for each other in a limbic memory mechanism, such that only their combined removal will yield an irrecoverable memory loss.

Confusion about the temporal order of events has sometimes been regarded as the fundamental defect in amnesia. Accordingly, it has been suggested that amnesia might reflect a selective deficit in assigning context to items that are to be remembered. An alternative hypothesis is that in normal memory as well as in amnesic material, contextual information is simply more fragile than other kinds of information.

We tested the patient N.A., who has chronic amnesia for verbal material, a group of psychiatric patients receiving bilateral electromcoagulation therapy, and matched control patients. Subjects read two lists of sentences 5 min apart, and then at varying times afterwards were given a yes/no recognition test and were also asked to judge which of the two lists the sentences had appeared on. For all cases, contextual information was deficient in amnesic patients. However, when the recognition memory of control Ss had declined as a result of forgetting to a point where it was equivalent to the recognition performance of amnesic Ss, contextual judgments of control Ss declined such that they matched the levels observed in amnesia. Thus, contextual information is fragile in amnesic patients, but it is fragile as well in normal Ss during the course of forgetting. The evidence suggests that the difference between normal and amnesic Ss may be understood in a quantitative way, and may resemble the difference between normal Ss tested shortly after learning and normal Ss tested long after learning.


Previous results (Gold and Sternberg, Science, 201, 367-9, 1978) indicate that when injected 30 min before training, the α-adrenergic antagonist, phenoxybenzamine (2 mg/kg) or the β-adrenergic antagonist, propranolol (0.5 mg/kg), attenuated retrograde amnesia produced by most classes of treatments (i.e., supra-seizure frontal cortex stimulation, sub-seizure amygdala stimulation, cycloheximide, pentylenetetrazol, and diethylaminocarbamate). The present study employed several adrenergic antagonists in order to assess the generality of these findings.

Animals were trained on either a one-trial inhibitory (passive) avoidance task or a visual discrimination avoidance T-maze. Thirty min prior to training animals received an injection of the β-adrenergic antagonist propranolol (0.5 mg/kg), saline, or one of the following α-adrenergic antagonists: phentolamine (10 mg/kg), piperoxane (5 mg/kg), or phenoxybenzamine (2 mg/kg). Shortly after training, the animals received frontal cortex stimulation (5 mA/1 sec) through implanted cortical screw electrodes. The results indicate that pretreatment of rats with any of these α- and β-adrenergic antagonists attenuates the production of retrograde amnesia.

In addition, we examined the effects of α- and β-adrenergic antagonists on amnesia produced by sub-seizure amygdala stimulation. Thirty min before training, animals received injections of either phenoxybenzamine (2 mg/kg), propranolol (0.5 mg/kg), or saline. Animals were trained on either of the tasks described above. Shortly after training, the animals received electrical shock of the amygdala (60 µa, 100 Hz, 0.1 msec monophasic pulses, 10 sec train duration) through bilaterally implanted electrodes. Twenty-four hours later, the animals were tested for retention. Consistent with the findings described above, pretreatment with the α- or β-adrenergic antagonists resulted in attenuation of amnesia produced by amygdala stimulation in both learning situations.

Thus it appears that several adrenergic antagonists can attenuate the amnesia produced by different treatments. These findings add further support to the view that there may be a common adrenergic mechanism underlying retrograde amnesia produced by many amnesic treatments.

Supported by research grants NS-11558 (NIMH) and NS-11558 (NIMH).


Anticholinergics have a long history of use in the treatment of symptoms of Parkinson's disease and are currently in common use either alone, or more often in combination with other antiparkinsonian medications. Recent studies have suggested that the putative neurotransmitter acetylcholine (Ach) may also play an important role in certain types of memory function. Acute injections of central acting Ach agonists have been shown to facilitate performance on memory tasks, while acute administration of Ach antagonists appears to disrupt memory functions transiently, particularly those involved in acquisition of new information. In a double-blind, cross-over study the therapeutic efficacy of the anticholinergic, benztropine mesylate (Cogentin [R]) was compared of vehicle.

Thirty min before training, animals received injections of either phenoxybenzamine (2 mg/kg), propranolol (0.5 mg/kg), or saline. Animals were trained on either the tasks described above. Shortly after training, the animals received electrical shock of the amygdala (60 µa, 100 Hz, 0.1 msec monophasic pulses, 10 sec train duration) through bilaterally implanted electrodes. Twenty-four hours later, the animals were tested for retention. Consistent with the findings described above, pretreatment with the α- or β-adrenergic antagonists resulted in attenuation of amnesia produced by amygdala stimulation in both learning situations.

This suggests that several adrenergic antagonists can attenuate the amnesia produced by different treatments. These findings add further support to the view that there may be a common adrenergic mechanism underlying retrograde amnesia produced by many amnesic treatments.

Supported by research grants NS-11558 (NIMH) and NS-11558 (NIMH).


Recent studies (Neurosci. Abstr. 1: 241, 1977) showed that phase-shifting circadian rhythms shortly after passive avoidance training can produce retrograde amnesia in rats. In order to further analyze this phenomenon, the effects of shock intensity and ACTH 4-10 (Org OI 63) were studied.

Male, albino rats were entrained to LD 12:12 prior to passive avoidance training. Rats were trained in a one-trial passive avoidance task with one of two levels of footshock (1.4 mA or 0.7 mA). Shortly after training, the LD cycle of phase-shifted groups was shifted by 12 h. Phase-shifted rats were housed in cages equipped for photocell activity recording. Activity records provided a measure of the progress of the phase-shift. Rats were tested 7 days after training, after the phase-shift was complete. One hour prior to testing, rats were injected with ACTH 4-10 (Org OI 63) (100 µg/rat, s.c.) or with an equivalent volume of saline, or one of the following α-adrenergic antagonists: phentolamine (10 mg/kg), piperoxane (5 mg/kg), or phenoxybenzamine (2 mg/kg). Shortly after training, the animals received frontal cortex stimulation (5 mA/1 sec) through implanted cortical screw electrodes. The results indicate that pretreatment of rats with any of these α- and β-adrenergic antagonists attenuates the production of retrograde amnesia.

As in earlier experiments, phase-shifting ciradian rhythms after training with 1.4 mA impaired retention performance (p<0.01). In contrast, phase-shifting ciradian rhythms after training with 0.7 mA facilitated retention performance (p<0.01). These differences in the effects of phase-shifting after different shock intensities suggest that phase-shifting alters memory by acting as a non-specific stressor.

ACTH 4-10 given 1 h prior to training facilitated retention performance of a 1.4 mA, phase-shifted group (p<0.01), suggesting that the disruptive effects of phase-shifting are due to retrieval effects. ACTH 4-10 further impaired the performance of a 0.7 mA, phase-shifted group (p<0.05). However, ACTH 4-10 did not facilitate the performance of a 0.7 mA, unshifted controls, suggesting that phase-shifting facilitates retention by reducing storage.

Phase-shifting appears to facilitate or disrupt memory as a result of its stressful consequences. However, phase-shifting appears to alter different memory processes, depending upon whether it facilitates (storage) or disrupts (retrieval) retention.
RESPONSES OF SINGLE HIPPOCAMPAL NEURONS DURING CLASSICAL CONDITIONING. Richard F. Thompson and Theodore W. Berger. Dept. of Physiology, Univ. of California, Irvine, CA 92717 and Dept. of Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260.

Responses of over 130 single hippocampal neurons recorded during classical conditioning of the nictitating membrane reflex in the rabbit were examined. All neurons were first classified as showing antidromic (and thus identified as pyramidal) or orthodromic (and thus classified as nonpyramidal) activation of the A5-ntermicemental, A7 dorsal raphe, and A9 locomotor area. All neurons were then assessed for their latency to respond and their ability to respond to a single presentation of the conditioning stimulus. The majority of pyramidal neurons had a latency of 5-10 msec to respond, whereas the majority of nonpyramidal neurons had a latency of 20-30 msec to respond.

DELAYED RESPONSE LEARNING AND BRAIN BIOGENIC AMINES IN CATS. Luc Vachon, André G. Oberteck and James Everett, Fac. Med., U. Laval, Québec, CANADA, G1K 7P4.

In an attempt to determine the involvement of CNS biogenic amines in short term memory and learning processes, the effects of a delayed response task (DR) on norepinephrine (NE), dopamine (DA), serotonin (5-HT) and 5-hydroxy-indolactic acid (5-HIAA) concentrations were investigated in normal cats. Moreover, the effects of drugs on DA responses were evaluated. In one group of cats, training on the DR produced a marked increase in NE and DA concentrations during the first day of training. In another group of cats, training on the DR produced a significant decrease in NE and DA concentrations during the first day of training. The majority of cats that responded showed a marked increase in NE and DA concentrations during the first day of training. The majority of cats that did not respond showed a marked decrease in NE and DA concentrations during the first day of training.

SINGLE DIGIT FLEXION-EXTENSION IN THE MONKEY, CALLITHRIX JACCHUS. Thomas J. Tobias and Dao Tien Duong, C. and O. Vogt Institute for Brain Research, and IV Department of Anatomy, University of Dusseldorf Moorrenstrasse 5, 4000 Dusseldorf, West Germany.

In an attempt to determine the involvement of CNS biogenic amines in short term memory and training characteristics of spontaneous activity (i.e., complex vs. simple spike patterns, spontaneous rate, etc.), we studied the spontaneous firing patterns recorded during conditioning trials.

Clear correlations were found to exist between cell type as determined by orthodromic and antidromic classification and the above criteria. The majority of cells identified as pyramidal neurons (i.e., antidromically activated) had lower (<10/sec) spontaneous rates and showed complex mode of unit firing during spontaneous periods. Orthodromically activated cells tended to have higher (>10/sec) spontaneous rates and did not show complex modes of discharge. The majority of pyramidal neurons increased rate of discharge and showed patterns of unit firing that corresponded closely to the topography of the conditioned NM movement. A majority of cells showing orthodromic activation inhibited during conditioning trials. Of cells that could not be activated in any manner after footshock stimulation, less than 10% showed no decrease in spontaneous rates and showing complex modes of unit firing during conditioning trials. The specific pattern of increased unit activity seen for orthodromically activated cells was learning-dependent. That is, pyramidal cells in animals given unpaired control training showed little or no increase in firing rates during training, whereas the majority of pyramidal cells recorded from conditioning animals on the first day of training showed growth in the amount of unit increase across initial paired trials.

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1078 PAPAVERINE FACILITATES PASSIVE AVOIDANCE IN MICE.

Because of our interest in CAMP as a second messenger potentially mediating memory we decided to test the behavioral effects of the phosphodiesterase inhibitor, papaverine HCl.

A preliminary experiment with 8 female NIH/ma mice indicated a significant (p<.025) performance facilitation in passive avoidance. In a more complete study, 63 male mice of the same strain received a 1 mA 1.5 sec duration footshock after stepping through a guillotine doorway. This shock level was chosen so that undrugged animals showed an intermediate performance level in the 24-hr retention (latencies approx. 300 sec). Maximum latency was 600 sec. Depending upon group designations animals received a 50 mg/kg I.P. injection of papaverine HCl or an equivalent volume of the NaCl vehicle. Group P-20 was injected with drug 20 min prior to training. This time allowed animals to recover from the overly observable sedative effects of papaverine. Group P+0 received the drug immediately after acquisition. Group S+0 received saline. Group P+60 was given papaverine 60 min after acquisition. A drug side effect control group received the drug but no footshock (PNS+0).

All groups except PNS+0 showed significant learning (p<.001) as measured by increased retention latencies. However, groups differed in retention performance at latencies. Because groups differed in retention performance at latencies, we speculate that papaverine may facilitate memory consolidation and/or retrieval. Whether this occurs as a result of changes which did not receive ESSB.

24 hrs later rats failed to show spontaneous recovery as compared to rats that received no ESSB. 4) Conditional Discrimination. Food deprivation schedules and/or retrieval. Whether this occurs as a result of changes which did not receive ESSB.

1079 Pathophysiologic Correlates of Cognitive Function
Bruce T. Volpe* William Hirst* Michael S. Gazzaniga
(GSPHF,Fl). Division of Cognitive Neuroscience, Cornell University Medical School and Rockefeller University, New York,N.Y. 10021.

Two unusually impaired amnesic patients have been extensively evaluated using current methods of cognitive psychology, and their disorders have been subsequently correlated with computerized tomography (CT). Results of these paradigms revealed that the memory disorder rested in the processes underlying memory function. The first patient, a fifty-two year old man, was a dense retrograde and anterograde amnesia to be left with a devastating inability to remember the temporal order of events. Although she performed substantially below a control group when free recalling a list of unrelated words, her recognition for items in the list was unimpaired. However, her recognition for the temporal relation between these items was severely impaired, as evinced by her chance performance on the part of a task in which random queries probed the relative recency of previously presented items. Her CT scan revealed the clips from a successful repair of a ruptured pericallosal artery aneurysm. Reminiscent of the transient Korsakoff-like syndrome described in the post cingulotomy state, she suffered a qualitatively similar yet permanent injury. The second patient, a fifty-six year old woman, suffered from a dense retrograde amnesia and performed like case H.M. on traditional tests. Analysis on another level however, suggested that impaired transfer from short term to long term memory, commonly called consolidation block could not completely explain this disorder. Techniques, such as semantic ordering tasks and cueing, successfully and predictably facilitated her performance in the situation may be viewed more productively as a failure of information processing, either involving encoding or retrieval modalities. In spite of this profound cognitive impairment, she was found to retain no evidence of a posterior lesion. Her lesion was more likely suggestive of a posterior destruction of the hippocampus. In sum our evidence is consistent with the hypothesis that a variety of central brain structures and not necessarily the hippocampus are involved in the memory process. (Aided by USPHS Grant No.25643, and the McNight Foundation).

1080 RECALL IN AMNESIC PATIENTS AS CONTROLLED BY ACQUISITION STRENGTH.
C. Douglas Wetzel & Larry R. Squire. Psychiatry Dept., Univ. of California & Veterans Hospital, La Jolla, CA, 92161.

Amnesic patients benefit from partial information cues to the extent that they initially acquire information. After a single presentation of a word list, control subjects showed better recall with meaningful semantic retrieval cues, compared to recall with either a rhyme cue or a cue consisting of the initial two letters of the word. By contrast, amnesic patients performed poorly and showed equivalent retrieval under all cue conditions. This amnesic pattern of performance could be mimicked in the recall of control subjects who were tested one day after learning instead of 1 min after learning. In addition, amnesic patients given repetitions of the learning list could mimic the pattern of control subjects tested 1 min after a single list presentation. Thus, with greater acquisition strength, the amnesic patients showed the normal pattern of performance: superior retention of semantically cued words.

The results suggest that the pattern of performance in amnesic patients can be largely determined by the strength of initial acquisition. As in other studies of anterograde amnesia, apparently qualitative differences between amnesic patients and control subjects can often be understood as quantitative differences in memory strength.

1081 IMPROVEMENT OF MEMORY IN FOUR DIFFERENT SITUATIONS BY SELF-STIMULATION.
Norman White and Daniel Coulombe*, Dept. of Psychol., McGill Univ., Montreal, Canada.

Four experiments demonstrating the improvement of memory by contiguous, but non-contingent electrical self-stimulation of the brain (ESSB) are described. The fact that four learned behaviors of such diversity are affected in similar ways by post-training ESSB shows the generality of the phenomenon. The subjects in all experiments were rats with chronic electrodes implanted in the dorsolateral quadrant of the lateral hypothalamus; all bar pressed for ESSB at a rate of at least 180 responses in 5 min. In all experiments the rats were trained on a task in a test apparatus and were then allowed to respond 1000 times for ESSB in a different situation. For the testing task the training was done 24 hrs. later. 1) Secondary Reinforcement. 48-hour deprived rats were allowed to lick a drinking tube 100 times; each lick produced a 0.2 sec tone (paired condition). A control group heard tones randomly throughout the session and licked 100 times (unpaired condition). 24 hrs. later all rats were tested with a dry drinking tube; a cone accompanied each 1sec. The rearranged rats in the paired condition with immediate ESSB made significantly more licks to extinction than a group of rats trained in the unpaired condition with immediate ESSB or than a group trained in the paired condition that received ESSB 2 hrs. after training. 2) Conditioned Emotional Response. On day 1 rats were water deprived and placed into a black cage where they heard a tone for 2 sec; 2 they were placed into a white cage and given 2 tones with foot shock during the last 0.5 sec of each tone (paired). The control group was given 2 tones and two shocks randomly (unpaired). On day 3 each rat was placed into the black cage with a water spout. When 10 sec of continuous drinking was completed the tone came on for 10 sec. In 10 such trials drinking was suppressed by the tone in the paired/immediate-ESSB group, but was not suppressed in the unpaired/immediate-ESSB group or in a paired/delayed-ESSB group.

3) Acceleration of Food Deprivation. Rats were trained to bar press for food. They were given one session of extinction training, followed by ESSB which they obtained by running in a square-shaped runway. On a second extinction session 2 hrs. later the rats failed to show spontaneous recovery as compared to rats that ran for ESSB in the pre-optic area, and rats that received no ESSB. Discrimination between ESSB and non-ESSB deprived rats were given 12 massed trials in a T-maze. With a white card at the choice point, food was always on the right; with a black card the food was on the left. 24 hrs later a similar task, which bar pressed for ESSB after the 12 training trials made more complex choices in the T-maze than rats which did not receive ESSB.
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Bromocryptine treatment. Together, the data from the GBL and haloperidol did not reverse the action of bromocryptine or ergo-
tors was assessed by measuring dopa accumulation in selected re-
neurons induced by γ-butyrolactone (GBL) (Walters and Roth, Naunyn-
neurons are associated with caudate-putamen cell bodies and processes
in dopa levels in the caudate nucleus. Haloperidol (1 mg/kg)
activity in this model, i.e., to reverse the GBL-induced increase

tors in this model, i.e., to reverse the GBL-induced increase

1083

Bilateral, unilateral or sham locus coeruleus lesions were made stereotaxically in Wistar rats by local microinjection of 5,7-dihydroxydopamine (5,7-DHT) at the rate of 7.5 μg per side for 30 minutes. After the lesion, the animals were killed and the cerebral cortex and hippocampus were obtained bilaterally. The cerebellum was dissected at the midline for the chronic (cyclopentane) and acute (5,7-DHT) experiments. Animals were injected intraperitoneally with the drug once daily for 10 days (4 mg/kg was administered on the first day and 1 mg on subsequent days), or saline for sham operations. Two weeks after the lesion, the animals were killed and the cerebral cortex and hippocampus were obtained bilaterally. The cerebellum was then dissected at the midline and divided at the midline. For the chronic reserpine treatment experiment, rats were injected intraperitoneally with the drug once daily for 10 days (4 mg/kg was administered on the first day and 1 mg on subsequent days). Two weeks after the lesion, the animals were killed and the cerebral cortex and hippocampus were obtained bilaterally. The cerebellum was then dissected at the midline and divided at the midline.

1084

Some of the ergot alkaloids seem to possess dopamine (DA) ago-
act on the DA receptor. Yet their onset is slow and duration of action
prolonged compared to other DA agonists such as apomorphine. The
activity of various ergot alkaloids at the presynaptic DA recep-
tors was assessed by measuring dopamine accumulation in selected re-
Figure 1: Immunohistochemical localization of noradrenergic neurons in the dorsal raphe nucleus (Aghajanian et al., Brain Res., 153:169, 1978). To
determine the relationship of the adrenergic input to these neuron-
types, we have undertaken the localization of adrenergic terminals
at the ultrastructural level by EM (electron microscopic) autoradiography.

1085

The dorsal raphe and the adjacent central gray receive a dense adrenergic input as demonstrated by biochemical, histochemical,
and immunocytochemical techniques. Previous pharmacologi-
cal studies have indicated that adrenergic terminals in the dorsal raphe
nerve terminals containing cells exert a tonic activating influence which maintains 5-HT cell firing activity (Baraban et al., Eur.
J. Pharmacol., 52:277, 1987). Recent studies have demonstrated
that non-5-HT neurons in the dorsal raphe nucleus function as mono-
aminergic terminals innervating non-5-HT neurons in the dorsal raphe.

1086

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1087

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aminergic terminals innervating non-5-HT neurons in the dorsal raphe.
1086 DOPAMINE (DA) AUTORECEPTORS: RAPID DECREASE IN THE RESPONSE TO DA FOLLOWING AGONIST TREATMENT. M.D. Barling, J.R. Walters, NIH, NIMCOs, Bethesda, MD 20005.

Single unit recording studies have demonstrated that spontaneously firing DA cells in the substantia nigra pars compacta (SNpc) recover from inhibition induced by a DA agonist such as apomorphine (AP). Apomorphine recovery pattern is different from, for example, the response of the serotoninergic raphe cells to LSD. After an i.v. dose of AP, 0.1 mg/kg, DA cell activity tended to be completely inhibited at 1 min and then recover rapidly to a plateau rate = 40% of baseline, from which further recovery is gradual (Bunney et al., Nature 245:123, 1973). Therefore, subsequent injections of AP (to follow the unstimulated period of inhibition and recovery to the plateau rate) until the cells are completely resistant to further injections of AP. At this point, apomorphine cells are also resistant to the normally induced inhibition of other dopaminergic agents such as pritendine, amphetamine (AMP), and L-dopa (Walters et al., Adv. Neuros. 8:272, 1979). We have more recently observed that cells resistant to the effects of AP are also no longer inhibited by either lergotrile (1.2-5 mg/kg) or tisuride (1.6 mg/kg). Two dopaminergic ergot derivatives. DAergic neurons in the SN and LC become resistant to the effects of this transmitter as the cells developed resistance to the effects of AP (cumulative dose, 0.1 mg/kg).

This agonist-induced resistance of DA autoreceptors might be a function of the partial antagonist properties of the drug. However, we have shown that the cells resistant to AP ini­ tiation of AMP, a drug which acts by releasing DA, and AMP inhibition, (cumulative dose, 12 mg/kg) show diminished responses to AMP, injected i.v. 15-45 min after AMP stimulation. A subsequent injection of AMP at 2.5 Hz produces about 50% of this maximum response while AMP stimulation at 1 Hz produces about 50% of this maximum response while AMP was injected i.v. 15-45 min before the subsequent injections of AMP. AMP stimulation of DA cells is heterogeneous with respect to the amount of immunoreactive enzyme; (c) if the dilution of primary antibody. Incubation of sections with primary antibody and reaction with 0.01% v/v DAB and 0.003% v/v H2O2 resulted in increased staining directly proportional to incubation time. After 15 min, the staining was complete. The

differences in staining intensities were 3 min. In untreated rats, cells of the LC are heterogeneous with respect to TH intensity for TH occurred in the SN. We conclude: (a) the PAP method can, under rigorous conditions, be utilized for quantita­ tion of tissue sections and (b) that neurons of the ascending serotonergic pathway are heterogeneous with respect to the amount of immunoreactive enzyme; (c) the induction of TH elicited by reserpine occurs in all neurons in the LC; and (d) reserpine failed to increase TH enzyme protein in dopaminergic neurons of the SN.

(Supported by NIH Grants HL 07379, HL 19879, and MH 24285.)


Bilateral stimulation of the cervical sympathetic trunks (CST) of the rat at 10 Hz produces an increase in the activity of serotonin-N-acetyltransferase (NAT) in the pineal gland. The rate and magnitude of the increase are similar to those observed during the normal nighttime rise in NAT activity in intact rats (Bowers and Zigmund, Soc. Neurosci. Abst. 4:268, 1978).

We have now studied the time course of this increase in NAT activity at a number of different frequencies and with several different patterns of stimulation. In all experiments male albino rats were exposed to light during the dark part of their day-night cycle. The CST were stimulated bilaterally with currents twice those required to produce maximal exophthalmos of the ipsilateral eye. Pinnales were removed and frozen immediately after the cessation of stimulation. Enzyme values are given in units of pmoles of product formed per μgm protein in 20 min ± S.E.M.

5 Hz stimulation for 0.5, 1.0, and 2.0 hours produced as large an increase in NAT activity as did stimulation at 10 Hz. For example, after one hour of stimulation at 10 Hz, the NAT activity was 27.2 ± 2.1 while after stimulation for the same length of time at 5 Hz the activity was 26.0 ± 2.1. The NAT activity of unstimulated animals was 2.0 ± 0.1. This suggests that stimulation of the cyst at 5 Hz is sufficient to produce a maximal increase in NAT activity in the rat. Stimulation at 2.5 Hz produces about 50% of this maximum response while stimulation at 1 Hz produces 10% of the maximum response.

In addition to its sensitivity to the frequency of stimulation of the cyst, the NAT activity of the pineal gland is sensitive to the pattern of stimulation. For instance, stimulation of the cyst with a repeating pattern of 5 Hz for 2 seconds followed by stimulation for 8 seconds produced a 3-fold greater increase in NAT activity after one hour than did stimulation continuously at 1 Hz. This difference occurred in spite of the fact that both conditions of stimulation consisted of the same number of stimuli. In contrast, stimulation at 5 Hz for 1 out of every 5 seconds for an hour produced an increase of the same magnitude as did continuous stimulation at 1 Hz. These data suggest that different changes in the pattern of firing of the cyst can produce large changes in the biochemical response of the pineal gland. (Supported by a grant from the American Heart Association.)


We sought to quantitate the changes induced by apomorphine (PAP) using the peroxidase-antiperoxidase (PAP) method: (a) if the enzyme tyrosine hydroxylase (TH) is uniformly distributed among all neurons of the substantia nigra (SN) and the rat brain; (b) if the increase in activity and accumulation of the enzyme (induction) elicited by reserpine (JPHET:197:757, 1975) occurs in all or only a sub-population of LC neurons; (c) if reserpine will yield a different magnitude of enzyme induction in dopaminergic neurons of the substantia nigra (SN) despite the absence of changes in TH activity (ibid). We first sought to determine the induction conditions. Injections of PAP (50 μg) into the substantia nigra (SN) produce large increases in TH activity (first order kinetics) with respect to the amount of immunoreactive protein; conditions necessary for quantitation. Untreated rats were perfused after PAP injections and brains postfixed in picric acid-paraformaldehyde and embedded in paraffin. Five micron adjacent sagittal sections were taken through the LC and SN and immunostained. Staining intensity was measured by a TV image analysis system (B & L Ominoc) measuring integrated optical density in neurons of the LC and SN. Linearity of the response (pseudo first-order kinetics) was primarily dependent upon: (a) the concentration of the substrate diamonobenzidine (DAB); (b) the duration of incubation with DAB; and (c) the dilution of primary antibody. Incubation of sections with 0.015% w/v DAB and 0.003% v/v H2O2 resulted in increased staining directly proportional to incubation time. After 15 min the staining was complete. The

differences in staining intensities were 3 min. In untreated rats, cells of the LC are heterogeneous with respect to TH intensity for TH occurred in the SN. We conclude: (a) the PAP method can, under rigorous conditions, be utilized for quantita­tion of tissue sections and (b) that neurons of the ascending serotonergic pathway are heterogeneous with respect to the amount of immunoreactive enzyme; (c) the induction of TH elicited by reserpine occurs in all neurons in the LC; and (d) reserpine failed to increase TH enzyme protein in dopaminergic neurons of the SN.

(Supported by NIH Grants HL 07379, HL 19879, and MH 24285.)

MONOAMINERGIC SYSTEMS
EFFECT OF NEONATAL 6-HYDROXYDOPA TREATMENT ON $\alpha$- AND $\beta$-ADRENERGIC RECEPTOR BINDING AND ON BEHAVIOR. David B. Bylund and Walid O. Shala

The treatment of neonatal rats with the neurotoxin 6-hydroxydopa is known to decrease adrenergic innervation and norepinephrine levels in the cerebral cortex while increasing innervation and norepinephrine levels in the cerebellum. Since the number of central $\beta$-adrenergic receptors, and perhaps $\alpha$-adrenergic receptors, appear to be inversely related to changes in norepinephrine levels (Bylund, Adv. Exp. Med. Biol. 115, 153-162, 1979), we studied the effects of 6-hydroxydopa on $\beta$-, $\alpha$-, and $\alpha_{2}$-adrenergic receptor binding. In addition, we studied the effects of the treatment on behavior in order to evaluate it as an animal model of the childhood hyperactivity syndrome.

Neonatal rats were treated (s.c.) days 1,3, and 5 with either 40 mg/kg 6-hydroxydopa, or vehicle (ascorbic acid-saline). At 24-26 days their locomotor activity was measured. Subsequently, the adrenergic receptors were assayed in crude particulate fractions of cerebral cortex, cerebellum, midbrain and medulla-pons using $\beta$-{3H}-dihydroalprenolol, $\alpha_{1}$-{3H}-NB-410(1 and $\alpha_{2}$-clomazone. Norepinephrine levels were determined using HPLC with electrochemical detection.

The 6-hydroxydopa treated animals were significantly hyperactive as compared to controls using both the activity box ($F(1,32)=5.4, p<0.05$) and the open field ($F(1,31)=12.4, p<0.001$) methods. As expected, the apparent number of $\beta$-adrenergic receptors ($B_{max}$) was significantly increased in the cerebral cortex ($F(3,29)=3.2, p<0.05$). Thus, 6-hydroxydopa produced increases in hyperactivity, $\beta$-adrenergic receptor binding, and norepinephrine levels.

However, no change was found in the number of cortical $\alpha_{2}$-adrenergic receptors as measured by $\alpha_{2}$-{3H}-NB-4101 binding. Similarly, $\beta$-receptor but not $\alpha$-receptor binding was decreased in the cerebellum. Thus, the effects of 6-hydroxydopa on $\beta$-adrenergic receptor binding appear to be regulated by the endogenous levels of norepinephrine.

Supported in part by NSF Grant BNS 7824715.

DIFFERENTIAL RECOVERY OF REINFORCEMENT AND MOTORIC FUNCTION FOLLOWING UNILATERAL LOSS OF BRAIN DOPAMINE. Robert J. Carey, VINC, SY 125.

Rats with bilateral medial forebrain bundle electrodes which generated comparable rate-intensity functions for self-stimulation were administered intraperitoneal injections of 6-hydroxydopa (4 $\mu$g of a $2\mu$g/ml sol.) into the substantia nigra. The nigral injections produced a virtual complete loss of forebrain dopamine in the injected hemisphere. Initially, this dopamine depletion was manifested behaviorally by a selective and severe decrease in self-stimulation in the dopamine deficient hemisphere as well as in ipsiversive circling and diminished locomotor activity. Over the course of a two month postoperative period, however, self-stimulation obtained from the dopamine depleted hemisphere gradually recovered and became more sensitive to stimulation than the intact hemisphere. In contrast, the ipsiversive circling and reduced activity level persisted throughout testing. Furthermore, the motoric responses elicited by stimulation of the depleted and intact hemispheres became different. While the typical exploratory behavior was observed to accompany stimulation in the intact hemisphere, stimulation of the dopamine-reduced hemisphere produced ipsiversive circling. In addition, the rats 2 mg/kg d-amphetamine produced reliable ipsiversive turning but at the same time facilitated self-stimulation equally for electrodes in the intact and dopamine depleted hemispheres. These studies show that dopamine depletion produces persistent motor but not reinforcement deficits.
GUANOSINE 5'-TRIPHOSPHATE IS AN ENDOGENOUS COMPOUND IN THE RABBIT CEREBellar CORTEX WHICH "COUPLES" THE BETA-ADRENERGIC RECEPTOR TO ADENY- LATE CYCLASE. Thomas E. Cote*, Tai C. Chen*, and John W. Kebabian

Guanosine 5'-triphosphate (GTP) or guanosine 5'-diphosphate (GDP) usually repeated within the particulate material in fractionation of the rabbit cerebellum abolishes the sensitivity of the adeny- late cyclase activity to beta-adrenergic agonists. The addition to the particulate cerebellar material of either the soluble consti- tuents of the cerebellar homogenate or the exogenous guanyl nucleotides, GTP or GDP, restores the sensitivity to beta- adrenergic agonists. Utilizing high pressure liquid chromatogra- phy (HPLC) the amount of GTP and GDP in the soluble components of the cerebellar homogenate can be measured; these guanyl nucleotides can account for the restoration of the sensitivity to beta-adrenergic agonists. The endogenous nucleotides in the cerebellum were isolated with HPLC. Only the endogenous GTP and GDP were capable of restoring the coupling between the beta- adrenoceptor and adenylate cyclase activity; none of the other compounds isolated with HPLC were active. The effectiveness of GDP may reflect its conversion, during the assay of adenylyl cyclase activity. If ATP is used as substrate, approximately 75% of the exogenous [14-C]-GDP is recovered as [14-C]-GTP at the end of the assay of adenylyl cyclase activity. However, if AMP-P(NH)P is the substrate such conversion is negli- gible; under these latter conditions GTP, but not GDP, can restore sensitivity to beta-adrenoceptor agonists. A non-specific antagonist, (3-H)-dihydroalprenolol (DHA) identifies specific binding sites similar to the beta-adrenoceptor which regulates adenylate cyclase activity. Exogenous DHA does not affect either the number of DNA binding sites or the affinity of these sites for 1-isoproterenol. Furthermore, GTP does not cause a shift in the activation affinity of the adenylyl cyclase activity for 1-isoproterenol. In conclusion, the guanylic nucleotides GTP and GDP are endogenous constituents of the rabbit cerebellum which are essential for the functional "coupling" of the beta-adrenoceptor and adenylyl cyclase but which do not affect the receptor per- se: the activity of GDP occurs as a consequence of its conver- sion into GTP; the activity of GTP may reflect a direct effect of this compound.

HORseradish peroxidase studies of an autonomic ganglion.

To further characterize the organization of the superior cervical ganglion (SCG), target organ and an efferent nerve of the rabbit SCG have been treated with horseradish peroxidase (HRP). The superior cervical ganglion was studied by light microscopy 4 hr after injection of HRP into the iris with HRP. Labeled ganglion cells seem to be evenly distributed throughout the SCG as seen with the o-dianisidine procedure for HRP. In contrast, injection of HRP into multiple sites in the submandibular sali- vary gland resulted in labeled neurons predominantly located in the caudal half of the SCG, near and below the origin of the external carotid artery. With the salivary gland injections, the external carotid or the external carotid nerve to HRP, labeled neurons were found in the rostral portion of the cervical sympathetic trunk, 4cm from the SCG at the level of the accessory cervical sympathetic ganglion and in the stellate ganglion. Tissue was also treated with diaminobenzidine for study of the distribution of horseradish peroxidase with the electron microscope. Lucensomes filled with HRP were found in the soma of ganglion cells as well as in neurites at the periphery of labeled cells. Some of the HRPs labeled neurites reached adrenergic terminals (shown by 5-hydroxydopamine loading) and terminals containing clear vesicles. In cases some nerves processes marked with HRP also contain scattered small dense core vesicles, although adrenergic terminals in the rabbit SCG have not been found to contain HRP.

These studies suggest that the superior cervical ganglion is not a homogeneous collection of neurons. Instead there is an apparent grouping within the SCG of neurons destined for a particular target organ. Neurons supplying the iris appear to be an exception to this pattern. The adrenergic fibers which are known to enter the caudal margin of the superior cervical ganglion arise from far caudal as the stellate ganglion. The recognized target organs of the superior cervical ganglion are the destination of some of these fibers.
1098 STANDARDIZATION OF THE SPG HISTOFLOUORESCENCE METHOD FOR MONOAMINE TRANSMITTERS. J.C. de la Torre, Dept. of Neurosurgery, Univ. of Miami School of Medicine, Miami, FL 33101.

The SPG histofluorescence method is a rapid, highly sensitive microscopic technique used to visualize tissue monoamine transmitters in cryostat sections. The SPG method differs from other histofluorescent microscopic procedures in 4 ways: 1. The reacting SPG solution sits at room temperature. 2. The tissue amine solution reaction takes 3 seconds. 3. The procedural treatment from frozen tissue to microscopic examination is done in under 10 minutes. 4. No elaborate equipment, animal perfusion or personnel trained in histology are required. Moreover, any amount of tissue can be wrapped in aluminum foil and buried in dry ice for transportation to the cryostat. One disadvantage in the original method (de la Torre & Surgeon, Histochemistry 49: 81, 1976) was the occasional variability in fluorescence intensity encountered after heating the tissue preparations on a hot plate during the first step in the procedure. The heating step has now been eliminated with the resulting effect of obtaining very consistent fluorescence preparations while still maintaining the high sensitivity of this previously noted methodology. The procedure follows: (a) Prepare the SPG solution: 10.2 g sucrose, 4.8 g monobasic KH₂PO₄ and 1.5 g glyoxylic acid monohydrate in 100 ml distilled water, pH 7.4 with 1 N NaOH and (b) top off with distilled water for a final volume of 150 ml. Prepare the tissue as follows: (1) Cut hippocampal sections 16-32 μm thick, pick up glass slides, and (2) Place slides 3 times (1 sec./dip) in SPG solution. Quickly rinse off excess solution from bottom and edges of slides before placing in acetone. (4) Stain placed between 2 strong hairpins at set maximum cool air for several minutes. Tissue must be completely dried on a ground glass appearance. (5) After drying, place tissue in groups of Light SPG solution. (6) Place slides in precooled 95°C oven for 2½ min. We recommend leaving a flat mineral oil to cover entire tissue on slide. (5) After drying, place 1-2 drops of Light USP mineral Oil on tissue, section is cover-slipped and examined in fluorescent microscope.


Studies in different animal models have established that cerebral ischemia profoundly alters the cerebral neurotransmitter firing patterns of the brain. In our present investigation, our model for ischemia consisted of mongolian gerbils subjected to a unilateral surgical occlusion of the common carotid artery. Since most of the cerebral cortex is supplied by the posterior communicating artery, the percentage of successfully induced ischemic subjects is directly related to the number of gerbils with this deficiency. Using the micropharmacological approach, we studied here the effects of drugs known to interfere with monoaminergic metabolism. One hour before the common carotid arteries was ligated in the gerbils anesthetized with ketamine 40 mg/kg i.p., niamid 100 or 200 mg/kg i.p., L.Dopa 300 mg/kg i.p., HTP (DL) 37.5 or 75 mg/kg i.p. or saline was administered intraperitoneally. Neurochemical studies were performed 24 hours after ischemia by McGraw et al. (Stroke 7:485-488, 1976) starting one hour after ligation and again at 4, 24, 48, 72 and 96 hours. Like saline, imipramine, niamid and L-Dopa had a selective effect on the gerbil's brain. The table summarizes the results obtained in treated groups compared to controls, p<.001).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n=4)</th>
<th>L-Dopa (500 mg/kg)</th>
<th>L-Dopa (100 mg/kg)</th>
<th>HTP (DL) 75 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPHE</td>
<td>3.7 ± 1.3</td>
<td>3.7 ± 1.3*</td>
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<tr>
<td>pPHE</td>
<td>9.8 ± 2.2</td>
<td>9.8 ± 2.2*</td>
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<td></td>
</tr>
<tr>
<td>MHPE</td>
<td>2.8 ± 1.3</td>
<td>2.8 ± 1.3*</td>
<td>2.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>L-Dopa</td>
<td>11.9 ± 2.8</td>
<td>11.9 ± 2.8*</td>
<td>11.9 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>HTP</td>
<td>3.7 ± 1.4*</td>
<td>3.7 ± 1.4</td>
<td>3.7 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

Mean Stroke Index at 96 hours.

+ significantly different from control.

1100 MICROIONTOPHORETIC STUDIES ON DENERVATION SUPERSENSITIVITY TO MONOAMINES IN THE RAT HIPPOCAMPUS. C. de Meurong, N. Mann, T.A. Reader and G.K. Aghajanian. Centre de recherche en sciences neurophysiologiques, Université de Montréal and Departments of Psychiatry and Pharmacology, Yale University, New Haven, Conn. 06520.

The dorsal hippocampus receives serotonin (5HT) and norepinephrine (NE) inputs from the median raphé and locus coeruleus respectively. The present study was undertaken to assess the susceptibility of hippocampal pyramidal cells of the CA3 region to putative neurotransmitters and analogs applied iontophoretically following damage to the raphe and NE pathways. A few microliters of 5HT (50 μg) was iontophoresized substances remained unchanged as compared with control values following 57DHT and 60HDA respectively. In 57DHT pretreated animals, the responsiveness of pyramidal cells to all iontophoresized substances remained unchanged as compared with control animals. As a test of the preservation of responses to ISO, 5HT and GABA were not altered by 60HDA pretreatment.

In a fourth group of animals the locus coeruleus was destroyed bilaterally by 5HTP (200 μg, free base) or 5,7-DHT (100 μg, free base) of one of the following substances: 1) 200 μg (free base) of 5,7-dihydroxytryptamine (57DHT) one hour after saline administration in 100 mg/kg, i.p.; 2) 200 μg (free base) of 6-hydroxydopamine (60HDA); 3) NaCl 0.9%.

In a separate experiment, a high dose of L-Dopa (500 mg/kg) injected 1 hour following the i.p. injection of 100 mg/kg of L-DOPA, brain levels of total MHPG, pHPG and MHPE were 93±4 (an increase of 53% compared to controls), while levels of free MHPG and pHPG in control rat brains (<2 ng/g) in control rat brains and could not be quantitated. One hour following the i.p. injection of L-Dopa treated animals, and if present was <0.6 ng/g. Our results suggest that L-Dopa is significantly metabolized in addition to NE and m-tyramine. These latter amines may therefore account for some of the effects of L-Dopa.


Although 3-methoxy-4-hydroxyphenylethanol (MHPE) is the major metabolite of norepinephrine in the CNS and its assay is often required as an index of NE metabolism, less attention has been paid to the alcoholic metabolites of other monoamines. They nevertheless appear to be quantitatively important metabolites of the parent amines, particularly the a-hydroxylated ones. We have developed a method using gas chromatography/mass spectrometry which permits the simultaneous assay of phenylethylamine, m- and p-hydroxyphenylethanol (mPHE & pPHE), p-hydroxyphenylglycol (pHPG), and 3-methoxy-4-hydroxyphenylglycol (HHPG). The metabolites of phenylethanolamine, m- and p-tyramine, octopamine and dopamine (DA), respectively, and HHPG and 3,4-dihydroxyphenylglycol (HHPG), both metabolites of NE, were obtained by gas chromatography/mass spectrometry in control and L-DOPA treated rats (300-350ng). These latter amines may therefore account for some of the effects of L-DOPA.

In a separate experiment, a high dose of L-DOPA (500 mg/kg) produced 15-, 59- and 4-fold increases in total pHPG and MHPE, respectively, and 4-, 6- and 7-fold increases in total MHPG. In a separate experiment, a high dose of L-DOPA (500 mg/kg) produced 15-, 59- and 4-fold increases in total pHPG and MHPE, respectively, and 4-, 6- and 7-fold increases in total MHPG.
Regulation of Tyrosine Hydroxylase Activity in the Dopamine Neuron After Severe Dopamine Depletion. D.C. German, B.A. McMillen, M. Dalgass, and P.A. Shore. Univ. of Texas Health Science Center, Dallas, TX 75235.

Tyrosine hydroxylase (TH) in dopamine (DA) neurons is activated not only by enhancement of impulse flow, but also by cessation of impulse flow. Enhanced impulse flow is believed to result in TH activation via removal of end product (DA) inhibition of the enzyme as well as by kinetic changes in TH. Cessation of impulse flow is believed to result in TH activation because of the absence of released DA to act on presynaptic DA receptors. Reduction of TH activity as measured after a large dose of reserpine (2.5 mg/kg s.c.) was used to assess the proliferative ability. TCA cells were not limited to rat gut. They were also found in mouse gut where they were present by 10 days’ gestation and disappeared before day 15. The TCA cells contain catecholamine and the biosynthetic enzymes, tyrosine hydroxylase (TH) and dopamine beta hydroxylase, for the dopamine group A2 in nucleus intercalatus. The shafts abutted bodies and dendrites of the adjacent reticular formation which extended from the cloaca to the bile duct on the dorsal surface of the gut. In recent studies, we have found that these nuclear bodies and dendrites inside Remak’s ganglion contain DA neuronal perikarya. It remains to be determined whether these immature DA neurons in Remak’s ganglion are adrenergic or cholinergic and whether they contain serotonin. These observations suggest that there are serotonergic neurons in Remak’s ganglion. Supported by NIH grants NS12969.
SLOW BURSTING NEURONS IN THE PREOPTIC/ANTERIOR HYPOTHALAMUS OF UNANESTHETIZED RABBITS: EFFECT OF NOREPINEPHRINE, SEROTONIN, AND THERMAL STIMULATION. C. J. Gordon* and J. E. Heath. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

Rabbits were stereotactically implanted with a guide tube to direct a microelectrode into the preoptic/anterior hypothalamus (POAH). A thermode and thermocouple reentrant tube to change and measure POAH temperature, respectively, and a cannula in the lateral ventricle to inject norepinephrine (NE) and serotonin (5-HT). A microdrive was secured to the head for lowering a tungsten microelectrode into the medial POAH. Firing rate of single units was recorded while the temperature of the POAH was clamped near 41°C and 34°C. When a neuron could be thermally classified, NE or 5-HT (30-100 μg) was infused into the lateral ventricle to inject norepinephrine (NE) and serotonin (5-HT).

A total of 55 single units were isolated in the POAH with 30% displaying a rhythmic bursting activity. The time ofbursting ranged from 9 to 67 seconds with a mean of 23±4 seconds. Three types of bursting neurons were isolated: (a) tonically firing cells induced into bursting by POAH thermal stimulation, (b) normally bursting units not influenced by POAH temperature but inhibited by NE (12% of bursting units) or 5-HT (19%), and (c) tonically firing cells induced into bursting by 5-HT (18% of bursting units) or NE (6%). In category "a" thermally induced bursting was inhibited by NE (12%) and 5-HT (19%).

Past investigations dealing with the measurement of thermally mediated motor responses have revealed rhythmic motor responses with frequencies similar to the neural bursting rhythms reported in this study. Occasional thermally sensitive bursting neurons may be important in the mediation of thermoregulatory reflexes.

Supported by NIH training grant H5N PHS GM7143.

Withdrawn by Author


A number of investigators using biochemical and electrophysiological techniques have reported the apparent excitatory effects of indirectly applied GABA agonists on dopamine (DA) cells of the substantia nigra. We further investigated the effects of GABA on substantia nigra neurons using single unit recording techniques and microiontophoresis. Both DA neurons and non-DA zona reticulata neurons were found to be inhibited by microiontophoretically applied GABA. However, a population of non-DA cells in the zona reticulata (ZR) was found to be 20 times more sensitive to the inhibitory effects of GABA than were DA neurons. GABA applied microiontophoretically in the vicinity of the ZR neuron caused an increase in activity of nigral DA cells recorded simultaneously. This effect was blocked by i.v. picrotoxin. Conversely, the excitatory substance glutamic acid, when iontophoretically ejected into the ZR caused an inhibition of DA neurons which was also blocked by i.v. picrotoxin. Dose response studies with the GABA agonist muscimol, administered parenterally (i.v.), revealed that incremental doses of muscimol increased the firing rate of DA neurons in a stepwise fashion which was directly proportional to the parallel inhibition this treatment induced in non-DA cells. Thus, GABA agonists appear to cause an increase in nigral DA cell firing rate by preferentially inhibiting GABA sensitive GABergic reticulata neurons (i.e. through a process of disinhibition).

This research is supported in part by U.S.P.H.S. grants, MH-28849, MH-25642 and MH-07527, and the State of Connecticut.

THE SPINAL PROJECTION OF THE LOCUS COERULEUS (LC) = AN ELECTROPHYSIOLOGICAL STUDY. P. G. Guyenet, University of Virginia School of Medicine, Charlottesville, VA 22908.

It has been reported in one recent histofluorescence study that all the noradrenaline (NE)-containing neurons of the rat LC may send a collateral into the spinal cord (SC); using retrograde labelling techniques, others have suggested that only a fraction of LC cells, located in the posterior and deep portions of the nucleus, send axons into the SC. In the present study, we confirm that even after multiple and massive pressure injections of HRP in the rat cervical SC (C5 - C7) only a subpopulation of LC cells, located as previously described, show evidence for the presence of the marker (2 - 3 μl total of 50% HRP, Harker-Tate reaction). This result prompted us to perform a comparative electrophysiological study of SC and other LC projections. Single barrel or five barrel electrodes were used for recording single unit activity in rats anesthetized with chloral hydrate and bipolar concentric electrodes to stimulate the SC (C5 - C7) or the dorsal NE bundle (DB).

A small minority of LC cells only could be driven antidromically (AD) from the SC with latencies of 25 - 50 msec corresponding to conduction velocities of 0.5 to 1 m/sec. Prominent A-B break was observed with paired stimuli. The refractory periods of the A and B segments were respectively 2 - 2.5 msec and 12 - 100 msec. These cells were spontaneously firing at 0.2 to 2.3 spike/sec and could be totally inhibited by iontophoretic morphine and GABA. Both DA neurons and non-DA zona reticulata neurons were found to be inhibited by microiontophoretically applied GABA. However, a population of non-DA cells in the zona reticulata (ZR) was found to be 20 times more sensitive to the inhibitory effects of GABA than were DA neurons. GABA applied microiontophoretically in the vicinity of the ZR neuron caused an increase in activity of nigral DA cells recorded simultaneously. This effect was blocked by i.v. picrotoxin. Conversely, the excitatory substance glutamic acid, when iontophoretically ejected into the ZR caused an inhibition of DA neurons which was also blocked by i.v. picrotoxin. Dose response studies with the GABA agonist muscimol, administered parenterally (i.v.), revealed that incremental doses of muscimol increased the firing rate of DA neurons in a stepwise fashion which was directly proportional to the parallel inhibition this treatment induced in non-DA cells. Thus, GABA agonists appear to cause an increase in nigral DA cell firing rate by preferentially inhibiting GABA sensitive GABergic reticulata neurons (i.e. through a process of disinhibition).

This research is supported in part by U.S.P.H.S. grants, MH-28849, MH-25642 and MH-07527, and the State of Connecticut.
ALTERATIONS IN [3H]CLONIDINE BINDING DUE TO 6-HYDROXYDOPAMINE AND MAO INHIBITORS. Margaret A. Hamburg*, Dorothy W. Gallagher, Iain Campbell*, and Edgar T. Talmage. (SPON: W. E. Bunney, Jr.) Biological Psychiatry Br. and Clinical Neuropharmacology Br., NIMH, Bethesda, MD 20205

[3H]Clonidine, a monoaminergic agent, binds specifically to membranes prepared from rat cerebral cortex. Binding is rapid, reversible and saturable. Initial studies suggested two binding sites for clonidine; however, a ten-fold dilution of membranes led to the appearance of a single set of sites with properties like the higher affinity site. In addition, when incubations are carried out in the presence of guanyl nucleotides, GTP and analogues decrease steady-state binding by enhancing the dissociation rate ten fold due to the elimination of a slowly dissociating component of binding. Thus, only in the presence of OTF does both rapid and complete dissociation of bound clonidine occur. These experiments indicate that several factors might be responsible for the appearance of the two sites. The resolution of two sites is a single site depending upon assay conditions is supported by data showing the pharmacological profile of the apparent two sites to be identical: αMT > piperoxane > yohimbine > WB4101 > prazosin. This profile is different from that of the WB4101 binding site: prazosin > WB4101 > yohimbine > αMT > piperoxane.

Lesions induced by intraventricular injection of 300 μg of 6-hydroxydopamine (6-OHDA) in increasing in the binding of [3H]clonidine in striatum. Scatchard analysis over a range of concentrations (0.25-40 nM) indicated a similar increase in binding at all points. WB4101 binding was also enhanced by 6-OHDA treatment.

Chronic (but not acute) treatment with the MAO Inhibitor clorgyline (4 mg/kg, s.c, daily) resulted in a 25% decrease in the potency of binding. Thus, only in the presence of GTP does both rapid and complete dissociation of bound clonidine occur. These experiments indicate that several factors might be responsible for the appearance of the two sites. The resolution of two sites is a single site depending upon assay conditions is supported by data showing the pharmacological profile of the apparent two sites to be identical: αMT > piperoxane > yohimbine > WB4101 > prazosin. This profile is different from that of the WB4101 binding site: prazosin > WB4101 > yohimbine > αMT > piperoxane.


Two weeks following unilateral locus coeruleus (LC) lesions all alocroduslices of rat brain were prepared. Striatal norepinephrine (NE) is depleted in the ipsilateral cerebral cortex of rats. NE levels in the cortical hemisphere contralateral to LC lesions were significantly different from those of control (sham lesioned) rats. NE depletion is associated with an increase in the density of α-α receptors as measured by the specific binding of [3H]-dihydroalprenolol. No changes, however, in α-α receptors are noted as measured by the specific binding of [3H]-norpseudoephedrine. This profile is not associated with loss of the normal blood volume increase when the cortical surface is stimulated to increased activity. The effects of electrically evoked dopamine β-3-reduction of cytochrome a,2 oxidized during the increased energy demand is slowed. The latter phenomenon occurs in striatal lesions in situ by dual wavelength reflectance spectrophotometry. In order to determine if compensatory changes occur in the depletion of NE and to relate these to the observed physiological index of β-NE receptor denervation supersensitivity, similar measurements were made 6 weeks following unilateral LC lesions. In these experiments, marked bilateral deficits were demonstrated in the cortical hemispheres ipsilateral to LC lesion. Despite the marked differences in NE levels between the two hemispheres, no differences were detected in the binding characteristics of α-α and β-NE receptors between the two sides. Likewise, increased energy demand in LC lesioned animals was accompanied in both hemispheres by increased blood volume and transient oxidations and re-reduction of cytochrome a,2. The rates of each of these reactions did not differ in the two hemispheres.

The return of the density of β-NE receptor binding sites, as well as the metabolic events, to conditions similar to those of the contralateral hemisphere, may be causally related or simply epi-phenomena of another event. In either case, it appears that the metabolic reactions described in an inhomogeneous physiological index of β-NE receptor denervation supersensitivity (Supported by Jack Wechter Memorial Fund)
LONG-TERM EFFECTS OF MULTIPLE DOSES OF METHAMPHETAMINE ON MONOAMINERGIC SYSTEMS

MONOAMINERGIC SYSTEMS

1116 MULTIPLE ELECTROCONVULSIVE SHOCKS INCREASE AMPHETAMINE-INDUCED ROTATIONAL BEHAVIOR BUT REVERSE ITS PREFERRED DIRECTION IN RATS. Gordon K. Hodge and Thomas L. Hall*. Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Although the mechanism responsible for the therapeutic effect of electroconvulsive shock (ECS) in depression is unclear, some behavioral data suggest partial involvement of catecholamine (CA) systems. Manipulations of CA systems in rats affect many behaviors in ECS treatment alters CA function, then it might be expected that ECS would produce commensurate behavioral changes in these animals.

To evaluate this possibility we compared effects of multiple ECS treatments (50 ma for 350 msec delivered across the ears; 1 treatment per day for 8 consecutive days; n = 14) with sham treatments (electrodes connected but no current; n = 14). 48 h before ECS treatments, all animals were administered d-amphetamine sulfate (2.0 mg/kg, i.p.), and rotational behavior was recorded for the following 3 h. At 48 h after the last ECS treatment, all rats were again given amphetamine, and their rotational activity recorded for 3 h. Body weights were measured on days of amphetamine administration.

Amphetamine-induced rotational behavior increased following ECS treatments (p<.05). Moreover, the preferred sides of rotation, as assessed by pretreatment directions, were reversed by ECS (p<.01). Posttreatment body weights of ECS-treated rats were lower than those of sham controls (p<.01).

ECS treatment undoubtedly creates profound alterations in many neural systems. But because it is therapeutically efficacious in reducing depression, and since drugs which facilitate CA function are also effective in this regard, it is possible that at least one mechanism for ECS effects involves enhancement of CA systems. Our results are consistent with this interpretation. Insofar as increased rotational behavior is indicative of a change in the increased rotations of the ECS animals may have been due to greater activity of CA systems; this is consistent with pharmacological studies in rats affecting which behavior in ECS treated rats was lower than that of control rats (p<.01).

Supported by U.S.N. Research, Administration grant 202-812-402.


Membrane binding of [3H]-5-hydroxytryptamine (5HT) was determined in crude homogenate preparations of whole rat brain minus cerebellum using the procedure of Bennett and Snyder (Molecular Pharmacology, 12: 316-326). Binding of fresh and freeze-dried tissue and tissue frozen immediately following decapitation were evaluated by tissue linearity studies in the presence of 337 nM 5HT and measuring the ligand concentration in the incubation medium. 100 μM unlabeled 5HT served as a blank for non-specific binding.

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<th>KD (μM)</th>
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<td>Fresh Control</td>
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<td>Frozen Control</td>
<td>4.34 ± 2.44</td>
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No significant changes were noted in pmoles 5HT bound/g using 7 nM 5HT ligand, in the equilibrium dissociation constant (KD), or in the number of receptor sites (Bmax) between fresh and frozen tissue. Because specific 5HT binding represented a higher fraction of total binding at 3.5 nM ligand concentration, this level was used in the remaining studies. To simulate human post-mortem conditions, rats were killed by cervical dislocation, allowed to remain at room temperature for 3 hours, and refrigerated for 16 - 18 hours; brain tissue was removed on ice and stored at -70°C for 24 hours or longer.

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<tr>
<td>Frozen Post-mortem</td>
<td>2.76 ± 0.90</td>
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Significant increases (p<.001) were observed in specific 5HT binding in post-mortem samples. An increase in the KD was also seen in tissue from rats treated to reproduce human post-mortem conditions. KD receptor density appeared to be reduced.

Membrane receptor binding of 5HT in homogenates of human post-mortem brain samples showed the following preliminary profile of regional 5HT binding: pallidum > precentral cortex, hypothalamus > postcentral cortex, thalamus > substantia nigra, putamen. Regional distribution was estimated as a function of the pmoles 5HT specifically bound per gram tissue. Regional distribution determined by Scatchard analysis of subcellular fractions of human post-mortem brain samples are in progress.

1114 A SIMPLE TECHNIQUE TO DEMONSTRATE MONOAMINE CONNECTIONS UTILIZING AXIAL TRANSPORT OF A FLUORESCENT DYE IN COMBINATION WITH THE GLYCYLIC ACID METHOD. Albert O. Humberton, Jr. and Walter R. BucE., Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

Recently several investigators have combined the HRP technique with the glyoxylic acid method in order to determine if any of the neurons contributing to specific projections contain monoamines. This combination technique, although laborious and time consuming, has overcome some of the difficulties by utilizing a fluorescent dye, 6,6-diamino-3-phenylindole (DAPI), in place of HRP as a retrograde marker. This dye facilitates the feasibility of using this system with the glyoxylic acid method by injecting the fluorescent dye into the cervical spinal cord. Two days following the injection the animals were sacrificed and the brains and spinal cords were processed with the glyoxylic acid method. The sections were stained with a 490nm filter system. We tested the feasibility of using this system with the glyoxylic acid method by injecting the fluorescent dye into the cervical spinal cord. Two days following the injection the animals were sacrificed and the brains and spinal cords were processed with the glyoxylic acid method. The sections were stained with a 490nm filter system in order to identify the monoamine containing neurons. The filter system was changed to 360nm. to identify the neurons containing the retrogradely transported DAPI. By merely switching the filter system back and forth, doubly labelled neurons can be identified. Details of the technique will be presented. (Supported by U.S.P.H.S. Grant MC-07410.)


Membrane binding of [3H]-5-hydroxytryptamine (5HT) was determined in crude homogenate preparations of whole rat brain minus cerebellum using the procedure of Bennett and Snyder (Molecular Pharmacology, 12: 316-326). Binding of fresh and freeze-dried tissue and tissue frozen immediately following decapitation were evaluated by tissue linearity studies in the presence of 337 nM 5HT and measuring the ligand concentration in the incubation medium. 100 μM unlabeled 5HT served as a blank for non-specific binding.

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Recently several investigators have combined the HRP technique with the glyoxylic acid method in order to determine if any of the neurons contributing to specific projections contain monoamines. This combined technique is useful, but somewhat difficult by utilizing a fluorescent dye, 4,6-diamino-2-phenylindole (DAPI), in place of HRP as a retrograde marker. This dye facilitated the feasibility of using this system with the glyoxylic acid method by injecting the fluorescent dye into the cervical spinal cord. Two days following the injection the animals were sacrificed and the brains and spinal cords were processed with the glyoxylic acid method. The sections were stained with a 490nm filter system in order to identify the monoamine containing neurons. The filter system was changed to 360nm. to identify the neurons containing the retrogradely transported DAPI. By merely switching the filter system back and forth, doubly labelled neurons can be identified. Details of the technique will be presented. (Supported by U.S.P.H.S. Grant MC-07410.)

Continuous exposure of rats to light increased the rate of dopamine turnover in retinal amacrine neurons and gradually elevated the apparent Vmax of retinal tyrosine hydroxylase (TH) over a 4 day period. Three different immunotitration procedures were performed on retinal homogenates to determine if the change in apparent Vmax was caused by a change in the amount of enzyme protein or a change in the specific activity of the enzyme. Antibody directed against rat adrenal TH (donated by T.H. Joh) effectively inhibited retinal TH activity. All three immunotitration procedures indicate that the change in apparent Vmax was due to a change in TH-specific activity rather than to a change in the amount of enzyme molecules. In addition, the change in apparent Vmax was found to be pH dependent, further supporting the notion of a change in specific activity.

Retinal amacrine neurons have dendritic processes but not axons. Our results suggest that changes in the specific activity of TH may be one of the mechanisms responsible for regulating catecholamine synthesis in dendrites.

CHANGES IN CATECHOLAMINE CONCENTRATION-TIME PROFILES IN HEART, BRAIN AND PLASMA AFTER ACUTE ISCHEMIC STROKE. NADER S. JALLAD*, DONALD J. WEIDLER, Division Of Clinical Pharmacology Department of Pharmacology, University of Miami, Miami, Florida.

We have demonstrated previously that the onset of acute cerebral ischemia causes myocardial damage and cardiac arrhythmias. Since the alteration of tissue and plasma catecholamine concentrations may be important in effecting these cardiac changes, the concentrations of norepinephrine, epinephrine and dopamine in heart, brain, and plasma were studied as a function of time for 24 hr after the onset of acute ischemia. Twenty-four anesthetized cats underwent ligation of the left middle cerebral artery, after which cats were permitted to awaken. Plasma samples were taken hourly and 4 cats each were killed at 4, 8, 12, 16, 20 and 24 hr. In brain tissue, norepinephrine and dopamine concentrations became significantly depleted in the ischemic cerebral hemisphere; but not in the non-ischemic hemisphere. The epinephrine concentrations in both ischemic and non-ischemic hemispheres did not change over the 24 hr. Plasma norepinephrine concentrations manifested two major peaks one at 8 hr and one at 20 hr. Plasma dopamine had a major peak at 8 hr and a minor peak at 18 hr, while plasma epinephrine peaked at 8 hr and 16 hr. In myocardial (left ventricular) tissue, the norepinephrine concentration was significantly elevated only in the 16-20 hr range; dopamine was elevated in the 4-8 hr range and peaked again at 16 hr. Myocardial epinephrine concentration had only one major peak, which occurred at 8 hr. The peak norepinephrine concentration in the heart (at 16 hr) was approximately 10 times the peak concentrations attained by epinephrine and dopamine. Based on these findings, we conclude that a period of increased susceptibility to cardiac arrhythmias may occur in the 16-20 hr period after the onset of acute cerebral ischemia.

In general, these data confirm previous studies of the action of these drugs on raphe neurons in immobilized and/or anesthetized rats. These results also provide general support for the serotonergic hypothesis of hallucinogenic drug action, since the four hallucinogens produced significant decreases in raphe unit activity, whereas amphetamine did not. However, since the potency of these various drugs in producing hallucinations in humans, and limb flicks in cats, i.e., LSD+DOM+5-MeO-DMT+Psilocin, does not correspond to their efficacy in depressing raphe unit activity, the latter effect apparently does not account for all of the important actions of hallucinogenic drugs. Aghajanian has shown that these drugs differ in their preferential action on raphe neurons as compared to their postsynaptic target neurons. Furthermore, several recent studies have shown that LSD and DOM have potent dopamine agonist effects; in addition to their effects on the brain serotonin system. Psilocin and 5-MeO-DMT, on the other hand, have little or no effect on the dopamine system. Therefore, we propose that inactivation of serotonergic neurotransmission is necessary and sufficient for hallucinogenic, and that additional dopamine agonist action greatly potentiates these effects.
1124 RELASE OF NOREPINEPHRINE AND DOPAMINE IN VITRO FROM BRAIN REGIONS OF AMYGDALOID KINDLED RATS. R. Jean Kant, James L. Meyerhoff, and Michael E. Corcoran. Dept. of Biological Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012, and Dent. Psychology, Univ. of Victoria, Victoria, B.C., Canada.

Goddard et al. reported that brief bursts of subconvulsive non-polarizing electrical brain stimulation presented chronically to different brain structures resulted eventually in a permanent change in the response of the animal to both localized seizure discharge, behavioral automatisms and generalized convulsions. This "kindling effect" has been shown to be trans-synaptic in nature, but the mechanism and specific neurons involved have not been identified. Considerable data indicate that brain catecholamine systems suppress various types of seizure susceptibility. It has been shown that drugs that potentiate norepinephrine and dopamine function suppress seizures while drugs that interfere with these monoamines can increase seizure susceptibility.

In our laboratory we have developed a sensitive model technique to measure small absolute amounts of endogenous norepinephrine and dopamine released in vitro. We decided to use this method to determine whether amygdaloïd kindling involves permanent changes in the releasability of NE and/or DA from various brain regions.

Thirty-four male Long-Evans rats were stereotactically implanted with bipolar electrodes into the basolateral amygdala. Seventeen animals were kindled beginning 10 days after surgery. Each day the rats received 1 sec of constant current 60 Hz square wave stimulation. The other 17 rats were sham-kindled and sacrificed 10 days after operation. 

Both spontaneous and KCl-stimulated release of NE and DA were similar for both kindled and control tissue in all tested regions.

1124 STRESS-INDUCED INCREASE IN DIIHYDROXYPHENYLACETIC ACID IN RAT FRONTAL CORTEX: MODIFICATION BY CHRONIC EXPOSURE TO COLD. Linda Kennedy*, Charles Saller*, and Michael Zigmond (SPON: B. N. DIXIT). Department of Pharmacology, School of Pharmacy, and Departments of Psychology and Biological Sciences, University of Pittsburgh, Pittsburgh, PA. 15260.

Rats subjected to restraint showed a significant elevation in plasma catecholamines 15 minutes after the onset of the stress (6-8 ng/ml peak vs. basal level of less than 2 ng/ml). This was accompanied by a 41% increase in dopamine (DA) (control: 0.06 pg/mg; restrained, 0.08 pg/mg; p < 0.05), and a 52% increase in dihydroxy-phenylacetic acid (DOPAC) (control, 0.07 mg/mg; restrained, 0.11 mg/mg; p < 0.05) in frontal cortex, suggesting an increase in DA turnover in this structure. No such change was observed in the striatum. The DOPAC concentration of frontal cortex was also an indicator of DA turnover in that structure. No such change was observed in 15mM KCl.

We next examined the ability of restraint to elevate DOPAC in animals subjected to chronic stress. Rats were maintained at 4°C for 15 days prior to sacrifice. Such treatment induced a 25% increase in tyrosine hydroxylase activity in frontal cortex (controls, 8.47 pmol/mg/mg protein/min; chronic cold, 10.38 pmol/mg protein/min; p < 0.05), but no increase in DOPAC level. However, in cold-stressed rats, 15 minutes of restraint produced a 94% increase in DOPAC, a three-fold larger increase than that observed following acute stress.

These data thus support previous suggestions that stress increases DA turnover in rat frontal cortex. They further suggest that while chronic stress enhances the behavioral response to subsequent acute stress. 

(Supported in part by USPHS grants~NIH-29670 and NIH-00058)


The Dorsal Raphe complex (DR) is a particularly complex region of the reticular formation which receives afferent fibers from widely scattered and functionally dissimilar regions of the CNS. Because of the great variety of these inputs, it is particularly appropriate to determine the degree of physiological convergence and divergence of the afferents within the Raphe.

In response to single shock stimuli delivered to the locus coeruleus (LC), substantia nigra (SN) and the inferior olive (IO), we recorded field potentials from within the DR. These recordings were made along a series of points from a set of electrode tracks which passed thru portions of the nuclei. After recording from a large number of points, we constructed a 4th dimensional matrix of data values which represented the rostral/caudal vs dorsal/ventral position vs voltage vs time response of the neuron.

The voltage measurements were converted to relative current according to the method of Howland et al. (2). Their algorithm eliminates the ordinary field potential recordings. Finally, these data were plotted as a time-sequence series of topographical contour maps with the peaks and valleys representing the sources and sinks of current respectively. Current sources are areas of net CNS depolarization.

Each of the three nuclei which were stimulated evoked rapidly developing (0.5 msec) current sinks which persisted as long as 7 msec. after the stimulus. One of these sinks, in the center of the nuclear complex, was common to all three stimuli. Other current sinks developed later (1.5 to 5.0 msec.) for each of the stimuli. In general, the LC evoked these later sinks in the caudal region of the DR followed by a very late developing, but intense sink in the rostral portion. The SN evoked additional sinks in the caudal, dorsal and rostral portions of the DR. The dorsal and rostral sinks overlapped the large rostral IC sink, but when activated earlier by the SN, some sinks associated with 10 stimuli were very complex, but were in general confined to the rostral part of the DR.


Supported by NIH Grant 5T27MH and The Tarbox Parkinson's Disease Institute.

Stimulation of the dorsal central gray area (DCG) and adjacent tissue elicits a variety of affective responses of fear and apprehension in humans and "fearlike" behavior in animals. Previous pharmacological studies have suggested that a serotonin (5HT) mechanism inhibits this fearlike behavior. The present study tested the hypothesis that the 5HT-containing dorsal raphe nucleus (DRN) is involved.

Data were obtained with two bipolar stimulating electrodes, one in the DCG and the DRN. The animals were trained to escape DCG stimulation (100 msec trains of capacity-coupled cathodal square wave pulses, 0.5 msec pulse duration, 60 Hz, 5 trains per second) by decremental bar pressing. In this paradigm, each bar press during a DCG stimulation period decrements the DCG current by 5% of its initial level. The animals received a baseline test run, consisting of twenty-five 120 second DCG stimulation trials (average initial DCG current = 20.4 ± 3.0 μA RMS) separated by 120 second time-out intervals (with no DCG stimulation). During the baseline test run, both the average number of decremental bar presses and the average latency to the first decremental bar press remained relatively constant. The follow-up day, the animals received an identical test run, except that DRN stimulation (100 msec trains of capacity-coupled cathodal square wave pulses, 0.5 msec pulse duration, 60 Hz, 5 trains per second) at 300 μA RMS was administered during the time-out periods preceding the first 15 DCG trials.

DRN stimulation caused a decrease in the average number of decremental bar presses, an increase in the average first bar press latency, and a profound reduction in the DCG fear-like behavior, yet did not affect the somnolence or other signs of peripheral impairment. This "anti-aversive" effect had a long latency to onset and persisted long after the cessation of DRN stimulation. Six days later, a test run identical to the baseline test run revealed that bar pressing returned to baseline.

These data indicate that stimulation of the DRN area reduces DCG fearlike behavior, possibly by influencing the DRN itself (or nearby endorphin-containing neurons). (Supported by NIMH grant MH-26032.)


When the cerebral cortex of rats is stimulated focally by trains of electrical square-wave pulses (2 sec trains, 0.5 msec pulse duration, 60 Hz, 5 trains per second) at 50.0 μA RMS was administered during the time-out periods preceding the first 15 DCG trials.

DRN stimulation caused a decrease in the average number of decremental bar presses, an increase in the average first bar press latency, and a profound reduction in the DCG fear-like behavior, yet did not affect the somnolence or other signs of peripheral impairment. This "anti-aversive" effect had a long latency to onset and persisted long after the cessation of DRN stimulation. Six days later, a test run identical to the baseline test run revealed that bar pressing returned to baseline.

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DEVELOPMENT OF MONOAMINERGIC CIRCUITRY IN SOMATOSENSORY CORTEX OF NEONATAL RAT. Donald A. Kristt and James D. Silverman*. Dept. Neurosci., Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In the present study three aspects of developing MA synaptic organization in postnatal neocortex were evaluated: (i) the distribution of MA synapses, (ii) the MA staining pattern with age and cortical depth, (iii) the structure of early formed MA synapses as compared to non-MA synapses, and (iii) differences among MA synapses as compared to non-MA synapses. Forty Sprague-Dawley rat pups, ranging in age from newborn to 16 days of age were studied. MA synapses were identified using an ultrastuctural anticytochrome oxidase marker, 5-hydroxydopamine (5-OHDA), which results in the appearance of small granular vesicles (SGV) in their pre-synaptic terminals. From birth to 7 days of age, 20-70% of all synapses, sampled in somatosensory cortex, contain 5-OHDA. However, few 5-OHDA synapses are seen in day 8 old cortex, and by 12 days of age, 5-OHDA's are no longer detectable in cortex. A specific distribution of MA synapses which is distinct from the overall synaptic distribution -- is first seen at 3 days of age and is essentially unchanged until 7 days postnatal. During this entire period, the 5-OHDA synapses predominate in the primordium of layer IV, where they account for 50-70% of all synapses. Morphometric analysis of 5-OHDA synapses indicates that there are differences in functional symmetry, vesicle shape and configuration of the contact zone between 5-OHDA and non-5-OHDA synapses, as well as between 5-OHDA synapses themselves in the various cortical layers. The laminar distribution and morphological characterization of 5-OHDA synapses suggest that the MA projection to neocortex exhibits a high degree of spatial specificity during its ontogenesis. A relatively high proportion of 5-OHDA synapses in the first postnatal week may reflect a potent influence exerted by the MA inputs on immature neocortex. The decreased neuronal density of MA neurons after 12 days of age is probably due to the development of the blood-brain barrier to 5-OHDA.

Although the origins of the pre-synaptic elements are not definite, it is established that these MA synapses are derived from MA nuclei in brainstem, and that they arrive in cortex before birth. Since this has not been directly tested, somatosensory cortex of several ages was injected with HRP. It was found that in the locus coeruleus numerous cells ipsilateral to the injection are retrogradely labeled before the 5-OHDA synapses become concentrated in the superficial layer IV. No neurons in the locus coeruleus in midbrain were labeled. A few neurons located in the region of C.N. VII were retrogradely labeled and may represent catecholaminergic neurons from cell group A5. Supported by a Teacher-Investigator Development Award NS-00279 from NIMH.


Voltammetric methods have been developed which allow continuous, simultaneous in vivo monitoring of the extracellular levels of dopamine (DA), 5-hydroxytryptamine (5-HT) and 3,4-dihydroxyphenylalanine (3,4-DOPA) in tissues of the rat brain. The extracellular concentration of DA in the striatum and nucleus accumbens of the rat is measured in situ by dual wavelength reflection spectrophotometry of cytochrome c oxidase activity, or by NADH fluorescence, using carbon electrodes. The technique is time-correlated with characteristic AMP-induced behavioral effects. Conversely, DA release with time course in correlation with drug-induced stereotyped behavior. DA release in the neocortex exhibits a high degree of spatial specificity during the ontogenesis. The laminar distribution and morphological characterization of 5-OHDA synapses suggest that the MA projection to neocortex exhibits a high degree of spatial specificity during its ontogenesis. The decreased neuronal density of MA neurons after 12 days of age is probably due to the development of the blood-brain barrier to 5-OHDA.

AMP, AMP and apomorphine were also used to investigate release of DA from SN. Release of DA was stimulated by AMP (5 mg/kg) and decreased both by AMP (250 mg/kg) and apomorphine (2 mg/kg). Pre-treatment with AMP (250 mg/kg) prevented AMP-stimulated release of DA.

These data indicate that in vivo voltammetry can be successfully employed to probe the functional dynamics of DA and serotonin neurons. Voltammetric recordings of MA activity may provide insights into the dynamics of MA release. (Supported by USPHS NIMH Grant NS-13556.)
GENESIS OF CENTRAL MONOAMINE (MA) NEURONS IN THE RHESUS MONKEY. Pat Levitt and Pasko Rakic. Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven, Ct.

Neurogenesis of the locus coeruleus (LC), substantia nigra (SN) and raphe complex (RC) was analyzed in autoradiograms from twenty-three to twenty-eight month old brains, each of which had been exposed to a single injection of 3H-thymidine on selected embryonic (E) days. Heavily labeled neurons, representing cells that had undergone final mitosis on the day of injection, were noted as early as E6 in 8 monkeys exposed to the isotope between E27-E36. LC neurons are generated between E27-36 with the majority proliferation occurring between E30-33. This same temporal series was also noted for neurons of the superior colliculus (SC) of the lateral geniculate body (LGB). However, although gestation in the rhesus monkey (165 days) is nearly twice as long as that in rodents, the major proliferation period of the LC occurs around E32. Cells that comprise the more diffuse areas of the LC are generated more evenly throughout the E30-33 period. SN neurons are generated between E28-43, with proliferation confined to two parts of the SN. Neurons of the ventral tegmental area are also generated between E38-43.

Neurogenesis of the RC occurs between E27-E43 with only a moderate rostrocaudal spatiotemporal gradient. Neurons of raphe dorsalis and centralis superior undergo final mitosis between E28-36, with the majority of labeled neurons being generated between E30-33. LC and SN neurons are generated between E27-36 with the major proliferation occurring between E30-33. The temporal series is consistent with that proposed for the development of the LC in the human being, although the timing of neurogenesis of MA neurons is different in these nuclear groups. The majority of neurons generated on E30 eventually become noradrenergic axons upon the development of cortical neurons or noradrenergic afferents to the developing telencephalon. Axons that synapse on the mesencephalon, junction of dopamine cells of origin of the mesolimbic and nigrostriatal systems with the medial terminal nucleus of the mesencephalon, contain antigens that are immunologically specific for dopamine axons. The effectiveness of the lesions was confirmed in adults by fluorochrome techniques, indicating that MA neuron systems in primates develop well into the last half of fetal life. (Lauder, et al., J Comp. Neurol., 155, 1974; Pierce, Proc. Br. Res., 40, 1973).

However, although gestation in the rhesus monkey (165 days) is nearly twice as long as in rodents (21 days in rat), and the number of MA cells several times greater, the majority of neurons of any single nucleus is generated within a similarly short period in both species (3-4 days in rodent; 3-5 days in macaque), indicating that MA neuron systems in primates develop rapidly from a larger precursor pool. (Supported by NS 14841).


Striated muscles of the stomatogastric system of many decapod crustaceans receive excitatory innervation from cholinergic or glutamatergic-type motor neurons. The effects of dopamine on muscles and neuromuscular junctions of the spiny lobster, Panulirus interruptus, foregut were examined.

Dopamine produces prolonged enhancement of nerve-evoked contractions down to at least 50 mV at both types of junctions. In these muscles receptors to cholinergic type of motor neuron, excitation occurred below 5 mV or less. In these two muscles dopamine also produces a contraction and frequently elicits spontaneous rhythmic contrac­tions that occur asynchronously in different fibers. The occurrence of such contractions just after removal of appropriate muscles from the animal suggests exposure to an endogenous agent able to elicit such actions. It is possible that dopamine, by underlying rhythmic contraction are endogenous to the muscles, but require tonic activity, is suggested by 1) asymmetry among adjacent fibers, 2) lack of effect of cholinergic receptor blocker tetraethylammonium (TEA) on contractions, and 3) failure of 1/10 Ca2+ to reduce spontaneous activity while evoked contractions are reduced. Octopamine and serotonin do not elicit these contractions, but reduce and increase, respectively, the frequency of their occurrence.

Intracellular recording from sensitive muscles during dopamine application reveals a slow depolarization and a conductance decrease consistent with a decrease in potassium conductance. A potassium conductance decrease is likely. Additionally, during dopamine application spontaneous rhythmic conductance increases are observed which may give rise to spontaneous contractions. Octopamine and serotonin do not appear to depolarize current pulses. A chloride conductance increase is likely. In muscles in which dopamine only produces enhancement of evoked contractions, effects are examined in control. Generally, small depolarizations are observed. Although effects on hyperpolarizing current pulses are small, dopamine produces an apparent conductance decrease when measured using depolarizing current pulses. The enhancement of sodium spike in the presence of dopamine can be accounted for entirely by effects on membrane resistance, and post-synaptic membrane conductance that is of action are likely. In addition, since the rate of relaxation of evoked contractions is increased during dopamine, a direct non-electrical effect on muscles has been excluded. These effects of dopamine appear to be unique to neuromuscular junctions of the stomatogastric system, since no effects of dopamine on Panulirus muscle evoked contractions were observed at 10 uM or below.

MONOAMINERGIC SYSTEMS


The rapid eye movements (REMs) of paradoxical sleep (PS) evoke light modulated potentials and multiple unit activity in the subthalamus and nigrostriatal systems that are abolished by lesions of the mesencephalon, junction of dopamine cells of origin of the mesolimbic and nigrostriatal systems with the medial terminal nucleus of the mesencephalon (Crespo, et al., Brain Behav. Evol. 60:541, 1978). Relationships observed between PS, photoperiod, maturation, and reproductive activities, led to the hypothesis that PS, REMs may serve to transmit photic information to regions of the brain which modulate cerebromonamine transmission in relation to daily and seasonal light changes.

To test this hypothesis the mesolimbic dopamine system excitability was examined in rats deprived of eye movements by personal muscle injection. Injection of low-dose amphetamine in the rat induces a marked increase in locomotor activity which depends on the integrity of the mesolimbic dopamine system. This response displays a circadian cycle reaching a zenith around midnight and a nadir around noon in rats on a 12:12 light/dark cycle. Twenty-two Long Evans rats, 11 eye muscle resected and 11 sham controls, were injected with 1.2 mg/kg d-amphetamine i.p. at 0100, 0700, 1100, 1500, and 1900 hours at intervals of not less than 28 hours and placed in activity cylinders equipped with photocells which recorded locomotor activity. Twelve weeks postoperatively, the normal relationship of the amphetamine-induced hyperactivity response to the daily light/dark cycle of operated rats was grossly disrupted compared with sham-operated controls. In two animals inadvertently blinded by the surgery, the circadian rhythm of the response was abolished. The data lend support to the hypothesis that lateral eye movements and REMs provide photic modulation to cerebral centers which regulate biologic rhythms of mesolimbic catecholamine activity.

1131 MONOAMINERGIC SYSTEMS

1130 NEUROTICAL DEVELOPMENT AFTER PRENATAL LESIONS OF NORDADERGENIC PROJECTIONS. Hart G.W. Lidove and Mark E. Mulliver, Departments of Cell Biology/Anatomy and Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Monoaminergic axons are amongst the earliest extrinsic afferents to invade the developing telencephalon and many of these axons synthesize catecholamines in the rat telencephalon as early as 13-14 days of gestation, while neurons of the cerebral neocortex are born and migrate from the ventricular zone during the first post-natal day. Furthermore, in perinatal cerebral cortex, presumably monoaminergic synapses constitute a large proportion of the total synaptic population (70% in layer IV of SC) and are immunohistochemically stained. Furthermore, since monoamine synapses can be distinguished by their sensitivity to 6-hydroxydopamine (6-OHDA) lesioning and dopamine-dehydroxylase immmunofluorescence; vehicle injected animals were used as controls. Material and method: Rats were exposed to excitotoxic injury on day 17 of gestation. Gestation was confirmed in adults by fluorochrome techniques, indicating that MA neuron systems in primates develop rapidly from a larger precursor pool. (Supported by NS 14841).

We have previously reported that serotonin is synthesized and stored in two locations in the lobster nervous system: the second thoracic roots and the pericardiac organs. Both these structures are characterized by a cortex of nerve ending-like varicosities with an appearance typical of neurosecretory tissues. In this communication we report that serotonin is released from these structures by depolarization and that physiologically effective levels of endogenous circulating serotonin are found in the hemolymph.

When second thoracic roots are incubated in H+-tryptophan they synthesize and store 3H-serotonin. This serotonin can be released in a calcium dependent manner by potassium induced depolarization. A five minute depolarization with 100 mM potassium will release about two-thirds of the newly synthesized serotonin in the root (1-2 moles of radioactive serotonin). When roots are incubated in the presence of the decarboxylase inhibitor NSD-1055, 5-hydroxytryptophan, the precursor of serotonin, accumulates instead of serotonin. Hydroxytryptophan is not released from these tissues with depolarization.

Serotonin levels in lobster hemolymph were measured using high performance liquid chromatography with electrochemical detection. A peak in the hemolymph samples was observed that eluted with the same retention time as authentic serotonin. This peak was further identified as serotonin by measuring the peak height as a function of the applied voltage of the electrochemical detector. We thereby determined that the compound in the hemolymph eluting at the same time as serotonin had oxidized properties similar to authentic serotonin. The basal hemolymph levels of serotonin in lobsters maintained in circulating sea water in holding tanks or in seminatural environments ranged from 10^-9 to 10^-8 M. In experimental animals, injections (.02-.2μl) of 30% Sigma Type A-V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

PROJECTIONS OF CONSTITUENT NEURONS. S.E. Loughlin*, S.L. Foote, and F.E. Bloom. A-V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

Neurons which project to a given terminal area are located within a few cases (e.g., neurons projecting to spinal cord, Satoh et al., Exp.Brain Res., 30,77, and others). In order to address this question in a systematic, quantitative, and computerized fashion we have been able to characterize 3-dimensional maps of the spatial distribution of LC neurons in rat and used them to determine whether, and to what extent, these neurons are topographically organized and whether this organization is reflected in their efferent projections. A computer-linked microscope is used to digitize LC cell positions and landmarks from serial sections. A computer graphics system is then used to re-create these sections and re-create a 3-dimensional LC. Initially, 15μ, sagittally oriented, Nissl stained sections from a paraffin embedded brain was used to reconstruct a "normal" LC. The total number of neurons digitized, approximately 1700, was in agreement with previous counts of the nucleus (Swanson, Br.Brain Res., 110, 76). In addition, computerized "sections" of the digitized LC in the frontal and horizontal planes yielded LC profiles that were similar to those observed in similarly oriented histological sections from other brains. The accuracy of this reconstruction is also being quantitatively assessed using statistical analyses which compare the number and distribution of cells in 2 or more normal nuclei. In experimental animals, injections (2-20 μl) of 30% Sigma Type A-V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

TOPOGRAPHICAL ORGANIZATION OF LOCUS CORONARIUS: EFFECTIVE PROJECTIONS OF DIFFERENTIATED NEURONS. S.E. Loughlin*, S.L. Foote, and F.E. Bloom. A.V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

The LC nucleus located in the ventral medulla oblongata in sympathetic ganglia, has been shown to have widely divergent efferent projections. The question of whether those neurons which project to a given terminal area is located within a few cases (e.g., neurons projecting to spinal cord, Satoh et al., Exp.Brain Res., 30, 77, and others). In order to address this question in a systematic, quantitative, and computerized fashion we have been able to characterize 3-dimensional maps of the spatial distribution of LC neurons in rat and used them to determine whether, and to what extent, these neurons are topographically organized and whether this organization is reflected in their efferent projections. A computer-linked microscope is used to digitize LC cell positions and landmarks from serial sections. A computer graphics system is then used to re-create these sections and re-create a 3-dimensional LC. Initially, 15μ, sagittally oriented, Nissl stained sections from a paraffin embedded brain was used to reconstruct a "normal" LC. The total number of neurons digitized, approximately 1700, was in agreement with previous counts of the nucleus (Swanson, Br.Brain Res., 110, 76). In addition, computerized "sections" of the digitized LC in the frontal and horizontal planes yielded LC profiles that were similar to those observed in similarly oriented histological sections from other brains. The accuracy of this reconstruction is also being quantitatively assessed using statistical analyses which compare the number and distribution of cells in 2 or more normal nuclei. In experimental animals, injections (2-20 μl) of 30% Sigma Type A-V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

VI HRP were placed stereotaxically in LC terminal areas and 40μl injections into hippocampus predominantly labeled cells in the dorsal hippocampus and septal areas. injections into amygdala resulted in several large, densely labeled cells scattered throughout LC and a greater number of lightly labeled neurons. The locations of labeled cells within LC following these HRP injections are also being mapped on the computer system. Distributions of labeled cells will be quantitatively assessed using statistical analyses which compare differences among these populations. USPHS Grant #AA03504.

Dibner and Mollinoff have reported that in vitro exposure of rat brain cortical slices to isoproterenol (150) causes a rapid and reversible decrease in \( \beta \)-receptor binding and activity. To further characterize this phenomenon, rat brain cortical slices were incubated at 37° in 10 mM Tris-HCl buffer containing \( \beta \)-adrenergic receptors were assayed using \( \beta \)-[\( \text{H} \)]-hydroxybenzylalcohol as a ligand; \( \alpha \)-adrenergic receptor binding was studied using \( \alpha \)-[\( \text{H} \)]-clonidine as a ligand. As reported previously, in slices incubated with 150 there was a 50-60% decrease in the number of \( \beta \)-adrenergic receptor binding sites occurring within 30 min, with no change in receptor affinity. However, in the present study, a 50-60% increase in \( \alpha \)-receptor binding was also found to occur in these membranes. The time-course of the \( \alpha \)-receptor increase paralleled the time-course of the \( \beta \)-receptor decrease. No change was noted in \( \alpha \)-[\( \text{H} \)]-clonidine receptor binding under these conditions.

In conclusion, \( \beta \)-adrenergic receptor changes induced by 150. In contrast, incubation with clonidine, a specific \( \alpha \)-receptor antagonist, resulted in a decrease in \( \alpha \)-receptor binding, with no change in \( \alpha \)- or \( \beta \)-receptor binding. This clonidine-induced decrease in \( \alpha \)-receptor binding was completely blocked by co-incubation with tolazoline (50μM), a specific \( \alpha \)-receptor antagonist.

These results suggest that there may be an interrelationship between \( \beta \)- and \( \alpha \)-adrenergic receptors such that overactivation of the former leads to desensitization of the \( \beta \)- and supersensitivity of the \( \alpha \)-receptor. This increase in \( \alpha \)-receptors may be a neuronal compensatory mechanism for overcoming enhanced \( \beta \)-receptor blockade.

Reference: Supported in part by USPHS grants NS-13803, an RCDA NS-00335 (J.J.E.) and a Salk Institute-Texas Research Fnd. Award.


In previous studies, we have demonstrated that various stresses (restraint, pinch, immobilization and cold) are associated with activation of rat adrenal tyrosine hydroxylase (TH). In the present study we have examined the effects of electroconvulsive shock (ECS) on adrenal and brain TH following both single and multiple (1/1x 7) ECS/day applications. Rats were shocked with 300 mA applied transorbitally for 0.2 seconds. Five or sixty minutes following the single application, and 24 hours following the multiple ECS, TH activity was assayed as described above.

The most powerful factor in the induction of TH activity was the administration of ECS to the animals. TH activity in the remaining 6 brain areas was unaffected by acute ECS treatment. Following repeated ECS treatment, TH activity was significantly increased in the adrenal gland, locus coeruleus and nucleus solitarius. Chronic ECS produced no change in TH activity in the remaining 5 brain areas.

Supported by USPHS grants NS 01919, NS 07972 and AA 03527.


Our previous results indicate that serotonin (5-HT) and norepinephrine (NE) markedly facilitate excitatory inputs to the facial nucleus without directly exciting facial motoneurons. (Brain Res., in press, 1979). In the present study the effects of hallucinogens on responses to 5-HT and NE on the facial nucleus were investigated by single-cell recording. Interventions such as d-lysergic acid diethylamide (LSD, 10-100 μg/kg) had no effect on the excitatory action of 5-HT and NE on motoneurons. In contrast, the facilitation of facial neuron excitation by antidopaminergic 5-HT and NE was greatly enhanced by low doses of LSD (5-10 μg/kg, iv.). The LSD-enhanced response continued for at least two hours. The long-term effect of LSD at low current which did not by themselves have any effect, also enhanced 5-HT facilitation at higher currents. Sustained response to 5-HT at higher currents was antagonized by the hallucinogenic 5-HT antagonist metergoline indicating that 5-HT receptors in the facial nucleus are unoccupied and that the LSD-enhanced response is due to the release of 5-HT from nerve terminals. The effects of two simple indoleamine hallucinogens, psilocin and N,N-dimethyltryptamine (DMT), were also tested. Like LSD, psilocin (0.5-2 mg/kg, iv.) markedly potentiated the effect of 5-HT and NE on motoneurons, but had no direct action when given alone. In contrast, DMT (0.5-2 mg/kg, iv.) facilitated glutamate-induced excitation of facial motoneurons by itself. This effect was blocked by 5-HT antagonist metergoline indicating that DMT can act as a 5-HT agonist in the facial nucleus. Like LSD, the hallucinogenic ergot derivative, lisuride, had no effect on glutamate-induced excitation of facial motoneurons. In contrast, a non-hallucinogenic ergot derivative, lisuride, had no effect on glutamate-induced excitation of facial motoneurons. In contrast, the facilitation of facial neuron excitation by a selective benzylophenothiazine 5-HT antagonist by itself was blocked (D-LSD-25 mg/kg, iv.) acted as a 5-HT agonist and potentiated the effect of NE. In contrast, a non-hallucinogenic ergot derivative, lisuride, had no effect on glutamate-induced excitation of facial motoneurons. In contrast, the peripheral 5-HT antagonists methysergide, metergoline, cyproheptadine and cimetidine blocked the action of DMT and potentiated the effect of NE. These data suggest that enhancement of certain spinal reflexes by LSD and mescaline (Andem et al., Br. J. Pharmacol. 1968a; 1968b; Macdonald, 29, 1977) may result from a sensitization of 5-HT and/or NE receptors in motor nuclei. In addition, if the sensitizing effects of hallucinogens on the facial nucleus occurs in other areas of the central nervous system, then the mechanism of receptor sensitization might contribute to the psychoactive effects of these drugs.
THE TIME COURSE OF DISCHARGE ACTIVITY OF LOCUS COERULEUS AND MIDBRAIN RAPHE REM-OFF NEURONS DURING ENTIRE SLEEP-WAKING CYCLES. Robert W. McCarley, Andrew Strassman, and J. Allen Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115.

There is increasing evidence that neurons in locus coeruleus (LC) and midbrain raphe may play a regulatory role in mammalian behavioral state control. To test the hypothesis that these neurons participate in control of the cyclically occurring events of the sleep cycle it is essential to know the time course of REM discharge activity over entire sleep cycles. Although previous studies had obtained samples of neuronal discharge activity distributed among various states, none had included entire cycle recordings. Therefore we decided to examine extracellularly recorded discharge activity during unperturbed, entire sleep cycles in unanesthetized, head-restrained cats. A particular aim was to compare the time course of REM discharge activity with that of REM-off activity predicted by the mathematical equations of this model.

For the experimental protocol, we used the technique of Blessing et al. (Neurosci. Lett. 9:311, 1978) to demonstrate catecholamine fluorescence and horseradish peroxidase (HRP) histochemistry following a single injection with a fluorescent agent. We have now examined the effects of PCA, a drug which produces a characteristic histochemical and fluorescence pattern correlated with the changes in histofluorescence observed following an amphetamine-like drug.

Although the noradrenergic innervation of the spinal cord has been well studied, the cells of origin of these pathways have not been well identified. We have used the technique of Blessing et al. (Neurosci. Lett. 9:311, 1978) to demonstrate catecholamine fluorescence and horseradish peroxidase (HRP) histochemistry following a single injection with a fluorescent agent. We have now examined the effects of PCA, a drug which produces a characteristic histochemical and fluorescence pattern correlated with the changes in histofluorescence observed following an amphetamine-like drug.
ROLE OF Dopamine STORAGE FUNCTION IN THE CONTROL OF Tyrosine HYDROXYLASE ACTIVITY IN THE Dopamine Neuron. B. A. McMullen and P. A. Shore. Dept. of Pharmacol., Univ. of Texas Health Science Center, Dallas, TX. 75235.

Cessation of nigro-striatal dopaminergic neuronal impulse flow by halothane or by reversal of stimulation of dopamine (GABA) leads to a marked increase in striatal tyrosine hydroxylase (TH) activity and in striatal dopamine (DA) content. Activation of TH is thought to arise from a decrease in the concentration of DA reaching the TH enzyme, which normally allows stimulation of pre-synaptic DA receptors (auto-receptors) which inhibit TH activity. However, TH activation following cessation of DA impulse flow or block of DA release was greatly elevated one day after reserpine, but had returned to normal by 3 days despite continued depression of DA levels. GBL enhancement of striatal DA storage function was greatly elevated one day after reserpine, but had returned to normal by 3 days despite continued depression of DA levels. GBL enhancement of striatal DA storage function was markedly reduced in 3 day reserpine rats compared with normal rats (1.2 vs. 3.7 μg/g above NSD-1015 alone). This effect appeared to be due to a shorter-lived effect of GBL on TH activity. Analysis of DA after GBL alone showed that GBL-induced increase in DA levels peaked at a short time and at a lower concentration than in controls (NSD-1015 alone).

The turnover of epinephrine (E) and the major catecholamines, noradrenaline (NE) and dopamine (DA) were studied in the cat. The functional significance of this overlap remains to be determined. On a relative basis, E turnover appears to be much greater than NE and/or DA in these regions. This high turnover rate in these brainstem areas suggests a possibly important role for E in the mechanism of a central response to stress.
Histochemical and immunocytochemical evidence in the literature has indicated that the remaining dopamine (DA) and norepinephrine (NE) in the mammalian olfactory bulb. The laminated structure of the olfactory bulb makes it possible to distinguish it from other enriched dopaminergic neuron types. By combining this with a very sensitive radioimmunoassay for DA, NE and epinephrine (E) (1), we have examined the subcellular localization of DA and NE in the following three catecholamine layers in the olfactory bulb: the fiber layer (F) containing incoming olfactory nerve fibers; the glomerular layer (G) containing mitral cell perikarya, glomeruli containing the axons of olfactory receptor neurons and mitral cell dendrites; and the inner granule cell layer (G) containing mitral cell perikarya, granule cells and the axons of mitral cells; and the white matter (W) containing afferent and efferent fibers of the olfactory bulb. The DA and NE exhibited distinct patterns in the fiber and granule cell layers as shown below.

<table>
<thead>
<tr>
<th>Catecholamine</th>
<th>Whole bulb</th>
<th>F</th>
<th>G or M-G</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA (ng/g wet wt.)</td>
<td>52</td>
<td>42</td>
<td>120</td>
<td>68</td>
</tr>
<tr>
<td>NE (ng/g wet wt.)</td>
<td>38</td>
<td>22</td>
<td>33</td>
<td>58</td>
</tr>
</tbody>
</table>

The levels of E were very low in the olfactory bulb (0.09 ng/g wet wt.) and showed a uniform distribution across the four layers and along the bulb. In whole bulb slices prepared from mice, we have obtained the following preliminary results: DA, 73 ng/g wet wt.; NE, 117 ng/g wet wt.; and E, 1 ng/g wet wt. The effects of peripheral deafferentation by unilateral removal of the LC projection by injections in areas causing generalized seizures. An operation after the injection, when the amount of the chemical is added to the input to the bulb had degenerated, the levels of DA in the mice treated with Zn or Vb were significantly lower (11 ng/g wet wt.) than those saline-treated controls (73 ng/g wet wt.). In contrast, the levels of NE and E in the bulbs of Zn- and Vb-treated animals were not significantly different from the saline-treated controls. Since DA levels in the olfactory epithelium are below the limits of sensitivity of the assay, it is possible to conclude that the origin of the DA which is lost is intrabulbar.

To determine the dependency of NE depletion on impulse flow in the LC projection, an electrothermic lesion was placed in the midbrain immediately before the intra-HIP KA injection, and the rats were sacrificed 3 hr later for NE assay. The bundle lesion alone in sham injected rats resulted in a slight increase in NE levels in the cortex; however, prior bundle lesion did not prevent NE reduction in any area within 3 hr of injection of 0.5 μg of KA into the HIP. The evaluation of the possibility that KA might act at presynaptic sites on the NE terminal, HIP slices were preincubated with [%]NE and the release of [%]NE was determined in a perfusion chamber. KA stimulated the release of [%]NE with an EC50 at 300 μM; release was blocked in Ca++ deficient medium. L-Glutamate was considerably more potent than N-methyl-NE (epinephrine). Both the alpha-adrenergic agonist, phenylephrine and the beta-adrenergic agonist, isoproterenol, were much less active than dopamine or phenylephrine. Synephrine, the N-methylated derivative of OCT, was considerably more potent than N-methyl-NE (epinephrine). Among the beta-adrenergic agonists, the OCT precursor, tyramine, was much more active than dopamine or phenylephrine. The effects of OCT were tested for their ability to block the activation of OCT-sensitive adenylate cyclase. These results support the presence of a phenylephrine receptor distinct from those receptors known to be activated by the catecholamines, dopamine, norepinephrine and epinephrine.
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SELECTIVITY OF NORADRENERGIC AXON SPROUTING FOLLOWING FETALAT
6-OHDA TREATMENT. John A. Gleimowa, Hunt G.W. Liddow, Reinhard Gramina, and Mark E. Hollings, Department of Cell Biochemistry, Anatomy and Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The administration of 6-hydroxydopamine (6-OHDA) or 6-hydroxypseudoephedrine early in development results in a marked elevation of norepinephrine, of (R) norepinephrine uptake, and of dopamine-beta-hydroxylase (DBH) activity. These occurred concurrently in brainstem and forebrain regions. DBH immunofluorescence revealed a total absence of NA axons in the telencephalon and a marked increase in the NA innervation of all brainstem nuclei. The results obtained by means of various neuroanatomical methods, this study emphasizes the complex and widespread distribution of NA collaterals. In rats, 6-OHDA was intraperitoneally injected on day 17 of gestation or during the first 3 days after birth. The whole injected side was restricted to those areas which normally receive a NA innervation. A marked increase was seen in the paraventricular, anteroseptal, and basal forebrain area. The thalamus, the area in the medulla oblongata, and in the sensory nuclei of V, and cochlear nuclei of the brainstem. Within cerebellar cortex, lobules VI and VII, area densely innervated in the normal and show the maximum degree of sprouting. The flocculo-nodular lobe, which is sparsely innervated in the normal, reveals very little sprouting. In both the cerebellum and in the thalamus of animals that normally receive a dense NA innervation, a significantly lower increase in NA fiber density than in sparsely innervated areas. In the cerebellar cortex regions, the density of noradrenergic fibers was increased in 6-OHDA-treated animals, the normal geometric pattern of NA axons was maintained. It can be concluded that the sprouting of noradrenergic fibers following 6-OHDA treatment is not random or diffuse, but exhibits a higher degree of neuron specificity than that which conforms to normal pattern of NA innervation. (Support: USNS NS06117, NS08513, NS10290; N.S.O.L. supported by training grant GM-7309.)

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Because of the possibility of their involvement in the acute or chronic effects of alcohol, certain tetrahydroisoquinolines (TIQs) derived from catecholamines (CA) and the AcH produced in vitro by alcohol dehydrogenase (ADH) were examined with respect to their metabolism and effects on amine synthesis in brain organotypic slices. These slices were prepared from alcohol-avoiding mice, which is in accord with the findings of others that the AcH produced in vitro by ADH has no effect on CA metabolism, whereas the AcH produced in vivo does not. Exposure of brain organotypic slices to AcH for up to 48 hr does not affect CA metabolism. The AcH produced in vivo was found to increase CA metabolism in vivo, and the AcH produced in vitro was found to decrease CA metabolism in vivo. The AcH produced in vivo was found to increase CA metabolism in vivo, and the AcH produced in vitro was found to decrease CA metabolism in vivo. 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ANALYSIS OF BIOGENIC AMINE METABOLITES IN RAT BRAIN HPLC WITH MORPHINE-TOLERANT PRIMATES AFTER TREATMENT WITH THE OPIOID ANTAGONIST, NALOXONE.

A novel finding is that naloxone may contribute to the abstinence syndrome in humans has focused attention on possible noradrenergic hyperactivity as a partial biological substrate2

Measurement of concentration of these metabolites can provide an index of the turnover of the neurotransmitter amines in brain. The high sensitivity of electrochemical detection (EC) compared to this hypothesis.2 We now present preliminary data showing in vivo uptake of 

The metabolites were determined in six brain regions (cerebellum, cortex, corpus striatum, hypothalamus, midbrain and brain stem) to demonstrate the utility of the method and the localization of the distribution of the metabolites. Typical concentrations (in pmol/g) in whole brain were 610 ± 20 for Dopac, 470 ± 20 for HVA, 2460 ± 60 for 5HIAA, and 2460 ± 60 for 5HIAA.

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It has been suggested that the characteristics of central adrenergic receptors may be regulated by the levels of noradrenaline (NE) at the adrenergic synapse and that this hypothesis is as well as to better define the presynaptic or postsynaptic location of adrenergic receptors in the brain, lesions of the dorsal NE tract from the locus coeruleus were induced by bilateral intracerebral injections of the neurotoxin 6-hydroxydopamine (6-OHDA). In eight brain regions of control and lesioned rats, the NE concentrations were 2.1 to 6.3 times higher in salivarectomized animals than in control rats. The results obtained in this study showed that the NE concentrations were increased in all brain regions studied.


Central norepinephrine (NE) pathways have been postulated to play a major role in the mediation of the hypertensive and behavioral effects of ethanol (EtoH). Recently, Mason et al. (1979) reported that 6-hydroxydopamine (6-OHDA) lesions decreased NE concentrations in the brain and that the reduction in NE concentration and the emergence of 15% v/v EtoH ingestion and blocked the reduction in locomotor behavior produced by 1.0 g/kg EtoH. In the present experiments the highest concentration (w/v) of EtoH preferred (at least 50%) of 24 hr fluid intake was established using the alternate-day free choice method in which each rat was exposed 3 times to 24 increments in EtoH concentration until they drank less than 40% of their daily fluid from the EtoH bottles. The EtoH concentration was then reduced until a preferred concentration was reached. Between rats these varied from 2-12% resulting in 1.0 - 5.4 g/kg/day EtoH intakes. The animals were exposed to the preferred EtoH concentration for 7 consecutive alternate days before and 9 days after bilateral NE lesions. The injection procedure

S. Ritter, B.C. Ritter and C.R. Christianson. College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

SUBMAXILLARY SALIVARECTOMY ELEVATES PLASMA CATHECOLAMINES. H.I. Yatnamura. Department of Pharmacology and Division of Biology, Loyola University, Chicago, IL 60657.

It has been suggested that the characteristics of central adrenergic receptors may be regulated by the levels of noradrenaline (NE) at the adrenergic synapse and that this hypothesis is as well as to better define the presynaptic or postsynaptic location of adrenergic receptors in the brain, lesions of the dorsal NE tract from the locus coeruleus were induced by bilateral intracerebral injections of the neurotoxin 6-hydroxydopamine (6-OHDA). In eight brain regions of control and lesioned rats, the NE concentrations were 2.1 to 6.3 times higher in salivarectomized animals than in control rats. The results obtained in this study showed that the NE concentrations were increased in all brain regions studied.

LATERALIZATION OF BEHAVIORAL AND CATHECOLAMINERGIC RESPONSE TO EITHER INFARCTION OR 6-HYDROXYDOPAMINE LESIONS OF THE CEREBRAL CORTEX IN RATS. Robert G. Robinson. Dept. of Psychiatric Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Right middle cerebral artery ligation in the rat leads to a 2 to 3 week period of spontaneous hyperactivity as compared with sham operated controls or preoperative baseline (Nature 255:322, 1975). Significant decreases in norepinephrine (NE) and dopamine (DA) concentrations in the cortex and brainstem of the unanesthetized rat immediately following 6-OHDA lesions in the cortex (Nature 255:322, 1975). In marked contrast, left middle cerebral artery ligation, although producing a comparable lesion, does not lead to hyperactivity. These animals are resistant to the pressor effects of EtoH. That the failure of such lesions to affect these measures may depend on the animals having been exposed to EtoH postoperatively is open to investigation. The present results, nevertheless, do not support the view that ascending DTB NE fibers play a significant role in mediating the behavioral effects of EtoH.

Supported by NIDA grants #DA02296.
MONOAMINERGIC SYSTEMS

1166 NOREPINEPHRINE ACTIVATES LATERAL GENICULATE NEURONS AND FACILITATES RETINAL INPUTS VIA AN α-ADRENERGIC RECEPTOR. Michael A. Rogawski and George K. Abajianian. Deps. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06510.

The dorsal lateral geniculate nucleus (LGN) receives a dense noradrenergic input from the locus coeruleus (LC). As has previously been demonstrated that electrical stimulation of the LC enhances the responsiveness of principal LGN neurons possibly by depressing inhibitory interneurons (Itoh and S. Tamura. Brain Res., 71:47-60, 1974). In the present study, the action of NE on LGN was examined directly using microiontophoresis in chloral hydrate-anesthetized cats and rats. Low iontophoretic currents of NE (1-15 nA) produced a delayed increase in the firing rate of most spontaneously active LGN neurons (latency 20-50 sec). This increase in spontaneous rate was mimicked by various sympathomimetic amines. The relative potency series of agonists was typical of postsynaptic α-adrenergic receptors: epinephrine > NE > phenylephrine > α-methylisopropylphenylephrine > dopamine (α-isopropyl). Prolonged (2-5 min) iontophoretic application of either d- or l-amphetamine, which release NE from noradrenergic nerve terminals, also activated LGN neurons. The α-antagonists phentolamine, piperoxane and n-4101 at low iontophoretic currents (<10 nA) produced a selective, dose-dependent and reversible blockade of the response to NE but not to glutamate. α-antagonist tachyphylaxis had weak and variable effects at equivalent iontophoretic currents. 

In order to examine the effects of NE on the response to different inputs, LGN neurons were orthodromically activated by electrical stimulation of the optic chiasm or with light flashes delivered above the eyes. The postsynaptic (r) component of the f wave response to photic stimulation which represents the mass activity of principal LGN neurons, increased in amplitude during NE iontophoresis. With subthreshold shocks, iontophoretic NE weakly enhanced the generation of both the early (<2 m sec) and late (100-300 m sec) components of the evoked response. NE also enhanced the responsiveness of LGN to light flashes. The increase in spontaneous activity was greater than the increase in spontaneous rate. 

It is concluded that NE activates LGN neurons via a postsynaptic α-type adrenergic receptor. Furthermore, these results suggest that NE released from axon terminals of coeruleo-geniculate neurons may serve to specifically enhance the transmission of visual information through the LGN. Supported by USPHS Grants MH 17871, MH 14459 and GM 7324 and by the State of Connecticut.


In the tail of the rhesus monkey caudate nucleus, which receives a projection from the LC, the discharge of single neurons has been found which respond with latencies of 90-140 ms to visual stimuli. The visual responses were selective, on the basis of the nature of the stimulus, e.g., such as orientation, colour or size. However, unlike the responses of neurons in the inferotemporal visual cortex, the responses of these were unaffected by prior visual exposure to the stimulus. The responses of these neurons diminished rapidly over the first 5 trials. Eye movement recordings showed that this occurred even though the animal was still fixating the stimulus. The evoked responses to visual stimuli were found to be mediated by dopamine systems. 

Thus the habituation was often to physically effective features. Arousal could not account for the responses of these neurons. In that the neurons responded only to a subset of arousing and non-arousing visual stimuli, and were relatively uninfluenced when arousal (as shown by the SGR) was induced by touch or by auditory stimulation. In the head and body of the caudate nucleus and in the nucleus acumens, different neurons were found, which responded either unconditionally to sensory stimuli, or conditionally to environment and reward. This difference in response to visual stimuli is related to the animal to prepare for the initiation of behavior, or throughout performance of a task, or in relation to particular movements (Rolls et al., 1979). Together, these different populations of striatal neurons could be involved in orienting behavior and in controlling the initiation and then enabling performance. Two possibilities follow. First, reduced function of these neuronal systems produced by dopamine depletion leads to a decrease in the incremental responsiveness or effectiveness of these neurons associated with over-effectiveness of the dopamine systems leads to such symptoms as inactivity or distractibility. Second, the generation of the smallest stimuli found in schizophrrenia which can thus be alleviated by neuroleptic drugs which block dopamine receptors.


The role of norepinephrine (NE) in oxidative energy metabolism of the cerebral cortex remains unknown. Such recent studies of the effects of the nucleus locus coeruleus (LC) discrete lesions produced by local stereotaxic microinjection of 6-OH-dopamine provide useful models for assessing NE actions. Such lesions were made unilaterally in Wistar rats (250-300 gm). Two weeks later, these rats were placed in a functionally fixed radiant heat source, artificially ventilated and holes were drilled in the skull, bilaterally exposing the frontoparietal regions. Oxidative metabolism was assessed by evaluation of changes in reduction/oxidation ratios of cytochrome α,α3 measured through intact dura by dual wavelength reflectance spectrophotometry. As in controls, hypoxia induced by changing the inspired gas mixture from normal (30% O2/70% N2) to 100% N2, was accompanied by increased cytochrome reduction. Transition from 30% O2 to 100% O2, or 95% O2/5% CO2 produced increased oxidation, indicating that the cytochrome oxidase redox state in LC lesioned rats is also partially reduced as in cats, rabbits and human neocortex. When ratios of oxidation to reduction produced by hypoxic and hypoxic transitions were calculated, no differences were apparent between hemispheres ipsilateral to LC lesion (NE depleted hemispheres) and contralateral hemispheres. These data demonstrate that if NE does have an effect on cortical metabolism, this effect is not apparent under "resting" conditions of low energy utilization. Differences did become apparent, however, when the brain was stimulated by electrical pulses delivered directly to the cortical surface. Both sides responded to such stimulation with excitation of visually effective effects. The reduction of cytochrome α,α3 back to baseline. However, in NE depleted hemispheres, the rate of re-reduction of cytochrome α,α3 was only partially allowed. These results indicate that NE plays a role in cortical energy metabolism under conditions of increased energy demand rather than under baseline conditions. Supported by NS 14319, NS 14325 and the Rita Cohen Memorial Fund.
brief spindle episodes (BSE) have previously been observed in the cortical electroencephalogram of DBA/2 and rarely in C57 mice, but not in C57BL/6 or BALB/c mice. BSEs are characterized by 1-5 sec bursts, with rapid onset and completion, of 6-7Hz monomodal waves which may attain amplitudes of 1200 μv or more. These episodes occur spontaneously and may be provoked by pentylenetetrazol.

Three lines of evidence suggest that the locus coeruleus (NE) system may be involved in modulating BSE activity.

1. Continuous recordings from 4 chronically implanted, freely moving DBA mice for a total of 168 hrs revealed that the average rate of BSE occurrence was 1.42 BSEs/hr. They primarily occur when the animal is awake and active, occasionally when drowsy and rarely during sleep. Catecholamine depletion by either reserpine (2.5 mg/kg, i.p.) or α-methyl-p-tyrosine (250 mg/kg, i.p.) produced a 6-50X increase in the rate of occurrence, beginning approximately 4 hrs after drug administration.

2. Eight chronically implanted DBA mice received 1.0 mg/kg mecamylamine, 2.0 mg/kg haloperidol and 10 mg/kg propranolol, presented in random order with 3 days between each drug presentation. During the 30 min recording after injection neither haloperidol nor mecamylamine affected BSE occurrence. After propranolol, BSEs occurred at a mean rate of 127/hr (31.2 SEM). In C57 mice, 10 mg/kg propranolol produced a few irregular bursts of spike and polyspike activity which may or may not be homologous to BSEs in DBA mice.

3. Acute transection of the brain stem of DBA mice caudal to the locus coeruleus (LC) had no effect on the occurrence of BSEs, and they could still be elicited by 10 mg/kg propranolol. Transection immediately rostral to LC resulted in BSEs. In 5 of 6 animals 116.4 (21.6 SEM) BSEs were recorded in the period from 1-1.5 hrs after transection. Physostigmine (0.25 mg/kg), given 1.5 hrs after transection of 3 of these mice, had no effect on BSE occurrence in the next 30 min, although this dosage was sufficient to produce cortical activation.

Thus, a beta blocking agent, propranolol, greatly exacerbates the occurrence of an EEG abnormality characteristic of DBA mice, whereas a dopaminergic blocking agent, haloperidol, does not. Transection of the ascending LC adrenergic tract also exacerbates the normality. It is interesting that DBA mice show a deficiency in cortical NE during development. This suggests that a genetic defect in the LC noradrenergic system of DBA mice is responsible for the appearance of BSEs.

[Supported in part by Council for Tobacco Research Grant #10767.]

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The effects of apomorphine (APO), a dopamine (DA) receptor agonist, on tissue glycogen levels were studied in rats, a brain region with a high dopaminergic innervation, and in the hippocampus, a region with very low DA levels and few, if any, DA receptors. APO (5mg/kg, s.c., injected 30 min. before insulin) decreased glycogen levels in the striatum and in the hippocampus (see table). Fluphenazine (FLU), a DA receptor blocker prevented these decreases, suggesting a role for DA in promoting glycogenolysis. Pretreatment of rats with propranolol (PROP), a nonnoradrenergic receptor antagonist, also prevented APO-induced glycogenolysis and, by itself, increased tissue glycogen levels. This result suggested a similar role for norepinephrine (NE). To determine if APO was acting by directly stimulating NE receptors or by indirectly activating NE-containing neurons, rats were depleted of NE and DA by administering reserpine (R) and α-methyltyrosine (AMT). This treatment elevated tissue glycogen levels and blocked APO-stimulated glycogenolysis. It was determined that the ability of all three pretreatments to block APO-induced glycogenolysis, only FLU blocked APO-induced stereotypy. Thus, APO may act through a DA system and while its glycogenolytic effects may require the activation of NE-containing neurons.

The glycogenolytic response to insulin was examined in rats pretreated with FLU (0.5 mg/kg, s.c.) or PROP (5 mg/kg, s.c.) 30 min. prior to receiving insulin (4-24 units, s.c.). Rats were sacrificed 30 min. after insulin and the insulin-stimulated 3H-glucose incorporated striatal glycogen by 441. This was unaffected by FLU, but completely blocked by PROP. Thus, the glycogenolytic response to insulin and to APO may be mediated by noradrenergic neurons.

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Quantitative evaluation of the change in permeability of the blood-brain barrier (BBB) to radioiodinated serum albumin ([351]H) by amphetamine in normotensive versus spontaneously hypertensive rats. When given prior to amphetamine that had previously been opened to permit the obtaining of a cardiac blood sample and was recorded. Fifteen minutes thereafter, the thoracic cavity was opened to permit the obtaining of a cardiac blood sample and was recorded. Fifteen minutes thereafter, the thoracic cavity was dissected out and the blood and brain samples were weighed and the subsequent perfusion through the heart with 30 ml of saline completely blocked by PROP (1mg/kg, s.c., 30 min.). Thus, a beta blocking agent, propranolol, greatly exacerbates the occurrence of an EEG abnormality characteristic of DBA mice, whereas a dopaminergic blocking agent, haloperidol, does not. Transection of the ascending LC adrenergic tract also exacerbates the abnormality. It is interesting that DBA mice show a deficiency in cortical NE during development. This suggests that a genetic defect in the LC noradrenergic system of DBA mice is responsible for the appearance of BSEs.

Data is expressed as the percent difference in glycogen levels between saline treated controls (striatum 1.93 ± 0.05 μmoles/g) and in hippocampus 2.51 ± 0.09 μmoles/g) and drug treated animals. These differences are significant (p<0.05) and hippocampus 2.51 ± 0.09 μmoles/g) and drug treated animals. These differences are significant (p<0.05).

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BEHAVIORALLY-DERIVED ESTIMATES OF CONDUCTION VELOCITY AND REFRACTORY PERIOD IN A ROD-COLUMNAR PATHWAY DIFFER FROM THE CHARACTERISTICS OF MONOAMINERGIC NEURONS. Peter Shizgal and Catherine Bielajew*, Dept. Psychol., Concordia U., Montreal, Que., H3G 1M8

When an axon bundle is stimulated concurrently at two sites, the orthodromic action potential (a.p.) from the upstream electrode collides with the antidromic a.p. from the downstream electrode and only a single a.p. reaches the synaptic terminals. If the time between stimulations at the two sites is sufficiently long, then collision will be avoided and both orthodromic a.p.'s will reach the terminals. The critical interval at which collisions are avoided will depend on conduction velocity (c.v.) and refractory period (r.p.).

Such collision effects can be inferred from behavior if the variables of stimulation are performed related to the frequency of firing in the stimulated pathway. (Collision reduces the frequency at which orthodromic a.p.'s arrive at the terminals.) Our study of the pathways mediating the rewarding and aversive effects of lateral hypothalamic (LH) and ventral tegmental (VTA) stimulation in the rat was based on this rationale. A frequency threshold scaling method (Teonians, Physiol. Behav., 15: 593-602, 1975) was used.

The principal findings and implications were as follows:

1. LH and VTA are directly linked to reward-related axons.
2. The estimated c.v.’s in these fibers range from 2.6 – 7.7 m/sec, values substantially higher than the reported c.v.’s of monoaminergic fibers.
3. Behaviorally-derived estimates of the r.p.’s of reward-related fibers are substantially shorter than the reported r.p.’s of monoaminergic fibers.

4. The finding of GTP regulation of agonist affinity for 3H-spiroperidol binding sites in the pituitary although it is inactive, despite the ability of these agents to compete for dopamine binding to receptors in vitro and to cause functional changes indicative of dopamine receptor stimulation in vivo.

Regulation of adenylate cyclase by GTP was first observed by H. Nusslein and co-workers in 1969. As a means of studying the adenylate cyclase system, a number of laboratories have used the pituitary gland, which is known to contain adenylate cyclase. Several laboratories have also focused on the human pituitary gland, which has a much higher concentration of adenylate cyclase than that of the rat gland. The majority of these studies have employed the intact pituitary gland and have utilized the hormone prolactin as a biological assay for adenylate cyclase activity. However, the pituitary gland is not a suitable means of assessing dopaminergic function, since the dopamine receptors in the pituitary gland do not respond to dopamine agonists acting at the level of the receptor. The dopamine receptors in the pituitary gland are distinct from those of the central nervous system, and the receptors of the pituitary gland are not sensitive to the action of dopamine agonists.

The finding of GTP regulation of agonist affinity for 3H-spiroperidol binding sites in the pituitary gland however, is consistent with the hypothesis that the pituitary gland contains dopamine receptors. This finding is also consistent with the findings of other laboratories that have investigated the regulation of adenylate cyclase by GTP in other systems. It is possible that the regulation of adenylate cyclase by GTP is a general property of adenylate cyclase, and that this regulation is mediated by a common mechanism. However, further studies are needed to determine the exact nature of this regulation and to determine whether the regulation is specific to dopamine receptors or whether it is a general property of adenylate cyclase.

Monoaminergic depletion of the central nervous system serotonin (5-HT), but not norepinephrine (NE), lowers jump thresholds to shock in the flinch-jump test whereas NE, but not 5-HT, depletes the maximal increase in paw-lack latencies in the tail-flick test (science Abstract 890, 1978). This stimulus-dependent hyperalgesia suggested that central 5-HT and NE systems modulate different aspects of nociception in the rat. The present study therefore examined the ability of four monamine reuptake inhibitors, with different relative selectivity for the 5-HT and NE reuptake mechanisms, to produce analgesia to shock in newborn and intact rats. Animals were injected with 10 mg/kg (i.p.) of the hydrochloride salts of fluoxetine (FLU), a highly selective 5-HT reuptake inhibitor, previously shown to increase jump thresholds (Messing et al., Psychopharm. Comm., 1975, 3, 511-521); nisoxetine (NIS) or desipramine (DMI), selective NE uptake inhibitors; or chlorimipramine (CMI), a mixed-acting uptake blocker. Vehicle-injected controls (VC) received saline i.p.

All four reuptake inhibitors produced analgesia, but the efficiency of each drug in altering pain sensitivity varied as measured by the assessment technique employed. FLU (+47%), CMI (+38%), and DMI (+24%) significantly increased jump thresholds compared to VC while NIS (+14%) did not. In contrast, DMI (+130%), NIS (+53%) and CMI (+45%) increased mean overall paw-lack latencies during testing at 30, 60, 120, and 180 min after injection while FLU (+41%) and VC evoked smaller increases at shorter time intervals compared to preinjection baseline. It was also found that FLU failed to produce analgesia on the hot-plate during a single determination (without preinjection testing) made 1 hr after injection. 5-HT reuptake inhibition is maximal. DMI increased latencies by 100% when tested only at 90 min after injection.

In a test of the neurochemical mechanisms of action of DMI in increasing paw-lack latencies, rats were depleted of either NE and dopamine (DA) by the intraventricular injection of 6-OHDA, or of DA alone, by the injection of 6-OHDA after DMI pretreatment. NE + DA depletion, but not DMI alone, blocked the analgesic action of DMI in the hot-plate test 30 days after surgery. The above data are consistent with the hypothesis that inhibition of NE, but not 5-HT, results in analgesia to noxious heat whereas 5-HT reuptake blockade is correlated with analgesia to shock. These studies therefore provide further evidence that 5-HT as well as NE normally suppresses the response to pain eventually but that their functions in nociception are dissociable.

Supported by USDA Grant No. 616681 and 616661.


Receptive fields for grooming reflexes (lick, bite, or scratch reflexes) are rarely seen in intact, adult cats or other species even though these reflexes can be elicited from newborns of some species (e.g., the rat). Previous work (Randall, Pharm. Biochem. Behav., 1974, 2, 355-360) indicates that the expression of the pons-midbrain tegmentum or of the frontal neocortex produces a 5-HT in hypothalamus(-71%), caudate(-61%), shell(-58%), and ventral tegmentum(-56%) in adult cats although p-CPA or ADX alone is ineffective (Randall, Elbin, & Swenson, J.C.F.P, 1974, 86, 747-750). p-CPA, however, is specific in that it also affects catecholamine metabolism, and when administered systemically, depletes serotonin peripherally. The present study shows that when central 5-HT levels are reduced by lesions of the superior central and dorsal raphe nuclei or by injection of 5,7-dihydroxytryptamine (5,7-DHT) into the superior colliculus, in conjunction with ADX, receptive fields for grooming reflexes appear that are the same as those seen in cats after p-CPA or ADX alone. grooming reflexes only occur after cortical replacement is discontinued after ADX. Single, systemic injections of 5-5-hydroxytryptophan (25mg/kg) or cocaine (100mg/kg) significantly reduced receptive fields whereas systemic administration of L-dihydroxyphenylalanine (50mg/kg) was without effect. In cats with the single treatment of either raphe lesion and 5,7-DHT alone, but significant receptive fields for grooming reflexes appeared only in the cats given 5,7-DHT. Raphe lesions led to significant reductions of 5-HT in hypothalamus(-51%), caudate(-57%), and substantia nigra(-38%) and superior colliculus(-38%) whereas, 5,7-DHT injection led to a significant reduction of 5-HT only in the superior colliculus (-53%). Thus, these data, along with the lesion and biochemical work, indicate that serotonin in the superior colliculus and systemic glucocorticoids are involved in inducing grooming reflexes in cats. (Supported by NIH grant # RO1 HS113402-07).

1181 NORDRENERGIC NEURONS IN THE LOCUS COERULEUS AND CARDIOVASCULAR FUNCTION IN NORMAL AND SPONTANEOUSLY HYPERTENSIVE RATS. Torgny H. Svensson, Goran Engberg* and Per-Anders Thörner, Dept. of Pharmacology and Cardiovascular Physiology, University of Göteborg, S-400 33 Göteborg, Sweden.

The pontine noradrenergic nucleus locus coeruleus (LC) has been claimed to participate in maintenance of apprehensiveness and arousal as well as in regulation of autonomic, e.g. cardiovascular function, involving various cardiovascular effects. Inhibition of the LC produces a pressor response and, recently, afferent stimulation of the vagus nerve was found to cause inhibition of LC neuronal activity. We have used single cell recording techniques to study the response of LC neurons in the chloral hydrate anesthetized rat and a physiologically relevant stimulus for cardiovascular reflexes, namely volume load. Intravenous injection of e.g. 1-4 ml blood caused a volume-dependent reduction in firing rate of LC neurons. Subsequent bleeding of the same amount of blood induced a pressure response to base line activity. Also bilateral vagotomy in the neck caused this reversal of the NA-cell inhibition. Thus, LC neurons respond reciprocally to moderate changes in blood volume, on effect probably mediated via vagal afferents. In spontaneous hypertensive rats (SHR) the average firing rate of randomly encountered NA neurons in the LC was progressively reduced with increased blood pressure of the animals when compared with normotensive, agematched Wister Kyoto rats (WKR). Bilateral vagotomy did not increase the reduced LC neuronal firing rate in SHR. The α-receptor agonist yohimbine produced a greater activation of LC neurons in SHR than in WKR suggesting possibly altered autoreceptor function at LC neurons. The LC NA neurons appeared biochemically equally active in awake SHR and WKR in contrast to e.g. nigrostriatal dopamine neurons (Rudolf et al., 1976). which seemed hyperactive in SHR. The LC NA cell activity may thus like heart rate and cardiac output in SHR be depressed below corresponding parameters of WKR controls after elimination of environmental stimuli by anesthesia.

Supported by the Swedish Medical Research Council (proj. nos. 4747, 4764 and 00016).

1182 EFFECT OF ANTIDEPRESSANTS ON NORDRENERGIC AND SEROTONERGIC RECEPTORS. S.W. Tang* and P. Seeman.(SPON. W. Brunelle, Pharmacology Department, University of Toronto, Toronto, CANADA).

In order to examine whether antidepressant drugs might act by inhibiting neurotransmitter receptors, the effects of these drugs were tested on noradrenergic and serotonergic receptors in vitro. The IC50 values or the concentrations of the antidepressants which 50% inhibited the binding of various 3H-ligands to a particulate fraction of the post-radiolabeled brain were determined. The antidepressants which 50% inhibited the binding of various 3H-ligands to homogenates of calf brain regions are listed in the Table.

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>3H-ligand</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>NMS-4101</td>
<td>20</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Clonidine</td>
<td>40</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Sero- tol</td>
<td>10</td>
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</table>

The two antidepressants of these drugs in the patient's plasma water is known to be of the order of 59-150 nM we conclude that:

1. Mianserin and the tertiary amine tricyclic antidepressants may block alpha-adrenergic transmission by blocking the postsynaptic alpha receptor (data using 3H-NM-4101).
2. Beta-adrenergic blockade is not inhibited by the drugs and thus may be enhanced by all antidepressants by either inhibition of noradrenaline re-uptake or by an increase in noradrenaline release (for the special case of Mianserin which inhibited the pre-synaptic alpha agonist presumably labelled by 3H-clonidine).
3. The weak but significant inhibition of serotonin receptors by some of the antidepressants may also contribute to the clinical antidepressant action.

(Supported by the Canadian Mental Health Foundation and the Medical Research Council of Canada).

We have recently reported that several psychoactive drugs such as haloperidol and amphetamine have significant effects on the development of central 5-hydroxytryptamine-containing neurons in the rat when administered during early postnatal periods (Gen. Pharmac. 9: 97, 1977; Pharmacol. Rev. 33: 109, 1981). We present data on the effects of neonatal treatment of rats with lithium chloride. Newborn Sprague-Dawley rats were given once daily subcutaneous injections of lithium chloride (LiCl, 1 mg/kg, s.c.) through a 4-6 day period. Control animals received injections of saline (1 pl/g) on the same schedule. No other treatment was given until either 25 or 60 days of age. At the rats were killed with L-5-hydroxytryptophan (5-MTP, 30 mg/kg, i.p.) or its vehicle and killed 60 min later. The rat brains were dissected into 10-12 regions and assayed for 5-hydroxytryptamine (5-HTP) and 5-hydroxyindoleacetic acid (5-HIAA).

Neonatal lithium treatment alone produced small (15-30%) but statistically significant alterations in brain 5-hydroxyindoleacetic acid levels. At 25 days of age the 5-HTP level was significantly elevated in the hippocampus, midbrain and thalamus and the 5-HIAA level significantly reduced in cortical cortex, olfactory tubercle and thalamus. The increased 5-HTP levels were no longer present at 60 days of age whereas the 5-HIAA levels were found to be significantly reduced in cortical cortex, olfactory tubercle and thalamus. As expected, 5-MTP injections at 25 and 60 days of age produced marked elevations in 5-HTP (200-400%) and 5-HIAA levels (200-700%) in all brain regions studied. However, the magnitudes of the 5-hydroxyindole increases were significantly affected by the neonatal drug treatment. At 25 days of age the 5-MTP-induced elevations in both 5-HTP and 5-HIAA were significantly larger (200-500%) in rats treated with LiCl during the neonatal period than in saline-treated controls in all brain regions except the hypothalamus. In contrast, the enhancement of the 5-MTP injections was no longer apparent at 60 days of age and in several brain regions (cerebellum, motor and temporal cortices, hippocampus, olfactory tubercle, parietal cortex) the increases were significantly less in the LiCl group. These data suggest that exposure to lithium salts during the neonatal period may alter the development of 5-HT-containing neurons in the central nervous system. (Supported in part by OMRF).


The dorsal bundle (DB) containing ascending noradrenergic (NE) fibers was lesioned in rats by bilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA). DB lesion decreased NE levels by 98% in the frontal cortex after one month. &-adrenergic receptor number, measured by &H-dihydroalprenolol (DHA) binding, increased 62% from 84.5 to 136.9 fmol/mg protein. The contribution of &beta1-receptor population, while &beta2-receptor number was unaffected by the lesion. Rats were also given &beta2-receptor antagonist practolol and the selective &beta1-receptor antagonist sotalol (30 mg/kg, p.o.) or 6-OHDA + sotalol, and then returned to normal food and water. Practolol or sotalol alone produced small (15-30%) but statistically significant decreases in 6-OHDA binding, and bound &beta1-receptor populations (100%) and &beta2-receptor populations (70%) in the DB-lesioned frontal cortex. The &beta1-receptor component of DHA binding accounted for 85% of total DHA binding, and the &beta2-component for 15%. Effect of both practolol and sotalol injection curves showed that the increase in cerebellar DHA binding was due to selective augmentation of the &beta2-population, while &beta1-receptor number was unchanged. Destruction of &beta2-receptor, thus causes an apparent increase in the number of &beta2-receptors in the frontal cortex, and of &beta1-receptors in the cerebellum. This suggests that functional neuronal supersensitivity of the &beta2-type in the frontal cortex and of the &beta1-type in the cerebellum. The location and significance of cortical &beta2-receptors and cerebellar &beta1-receptors is as yet unclear.

Supported by USPHS grants RR-05370, GM-30626 and GM-27527.


Tyrosine 3-monooxygenase (TYRO-3-M0) is a cytochrome P-450-dependent enzyme that catalyzes the conversion of tyrosine to dihydroxyphenylalanine and dopamine. In a transplantable rat pheochromocytoma, the carboxylic ionophores monensin, lasalocid and ionomycin are known to increase TYRO activity. We have recently reported that several psychoactive drugs as well as several neurotransmitters affect TYRO activity in these cells (Perlman, Fed. Proc. 38: 525, 1979). We have now examined the effects of these ionophores on TYRO activity in intact pheochromocytoma cells. TYRO activity was assayed by measuring the formation of and salbutamol binding were determined by a computer analysis using a TGGL program (Upjohn Co.). Salbutamol bound with high affinity (Kd = 0.2 μM) to 20% of the DNA frontal cortex sites, which represented &beta2-binding, and bound with low affinity (Kd = 4.0 μM) to the other 80% of sites, which represented &beta1-binding receptor. Conversely, propranolol bound with high affinity (Kd = 0.4 μM) to the majority (β1) population, and with low affinity (Kd = 10 μM) to the minority (β2) population of DNA cortex sites. Analysis of inhibition of DNA binding by either propranolol or salbutamol showed that the fraction of total sites represented by the number of β2-sites approximately doubled, whereas the number of β1-sites was unaffected by the lesion. All the receptor sites were also shown to be increased in the frontal cortex, and increased overall cerebellar DNA β-receptor binding by 35%. &Eacute;ado-Hoffstee plots of propranolol and salbutamol inhibition of cerebellar DNA β-receptor binding, contrary to the frontal cortex, the β2-component of DNA binding accounted for 20% of total DNA binding, and the β3-component for 80%. A decrease in the number of β3-sites may be responsible for the fact that the increase in cerebellar DNA binding was due to selective augmentation of the β2-population, while β1-receptor number was unaffected. Destruction of β2-receptors, thus causes functional neuronal supersensitivity of β2-receptors in the frontal cortex, and of β1-receptors in the cerebellum. This suggests that functional neuronal supersensitivity of the β2-type in the frontal cortex and of the β1-type in the cerebellum. The location and significance of cortical β2-receptors and cerebellar β1-receptors is as yet unclear.

Supported by USPHS grants RR-05370, NIH-30626 and NIH-27527.

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Following large doses of atropine SO4 . rats display large amplitude slow waves in the neocortex during immobility, tremor, tooth-chattering, and face-washing, (Type II behavior) but display atropine resistant low voltage fast activity (ARLVFA) during walking, struggling, postural changes, and head movement (Type I behavior). ARLVFA is abolished by prior treatment with reserpine but not by chlorpromazine, triffuuperazine, haloperidol, pinazide, \( \beta \)-methyl-\( \beta \)-tyrosine, \( \beta \)-chlorphenylalanine, methysergide, LSD or promethazine. However, since many of these drugs reduce the emission of Type I behavior, they produce a correlated reduction in the occurrence of ARLVFA. The effect of reserpine on ARLVFA is blocked by prior treatment with mialamide, suggesting the involvement of a monoamine. Replacement experiments showed that following reserpine, ARLVFA was not restored by \( \alpha \)-dopa, apomorphine, clonidine or \( \beta \)-hydroxytryptophan.

However, \( \beta \)-phenylethylamine (PEA) appeared to restore normal ARLVFA, together with Type I behavior. Restoration of ARLVFA was not affected by the administration of \( \alpha \)-methyl-\( \beta \)-tyrosine, trifluuperazine or chlorpromazine, although these drugs eliminate most PEA-produced behavior. PEA may play a direct role in ARLVFA independent of catecholamines.

Although \( \alpha \)-dopa and apomorphine were ineffective restoring ARLVFA in reserpine treated rats, a) an increase in Type I behavior and, b) an increase in atropine sensitive LWFa. Dopamine may play an indirect role in ARLVFA by stimulating the emission of Type I behavior and may also play some role in the atropine sensitive LWFa which is normally present during waking immobility.

(Supported by grants from the Natural Sciences and Engineering Research Council of Canada)


The presence of amineric fibers in both the superior colliculus and the lateral geniculate nucleus have been shown in the rat by using histofluorescence methods (Fuze, Acta physiol. scand. 64 (suppl. 247): 37, 1970). Since then effort has been done to demonstrate them with other techniques (Conrad et al., J. Comp. Neurol. 156: 179-206, 1974; Moore et al. J. Comp. Neurol. 180: 417-438, 1976) being the results controversial.

In this study the axonal transport of the horseradish peroxidase (HRP) was used to trace the afferents to the superior colliculus and the lateral geniculate nucleus. Injections up to 0.05 micro-liters of 30-50% HRP were made through micro-prociples. In addition iptomotrophic deposit of HRP was also performed to avoid diffusion of the marker to neighbor regions.

After 18-24 h of survival the animals were killed by perfusion through the heart with Karowsky fixative; then the brains were removed and processed according to a routine method (Pasquier & Reinoso-Sureda, Brain Res. 120: 540-548, 1977).

Large HRP injections in the superior colliculus labeled numerous neurons in the dorsal raphe nucleus, which were placed almost exclusively in its lateral wings. HRP deposit by iptomoteresis labeled, conversely, some neurons in the lateral wings of the dorsal raphe nucleus.

These results suggest, 1) that an amineric projection from the dorsal raphe nucleus reaches the superior colliculus and the lateral geniculate nucleus in the rat, and 2) that this projection is originated mainly from a lateral caudal extension of the dorsal raphe, which has remained largely ignored.


Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, catalyzes the conversion of tyrosine to DOPA. Apparent enzyme activity is changed by altering its affinity for the reducing agent, \( \alpha \)-dopa (Km) or the Mg ATP (Km), the end-product feedback inhibitor dopamine. In agreement with other laboratories, when cAMP and Mg ATP are included during phosphorylating conditions, there is an increase in the Kie for dopamine and no change in the apparent enzyme activity.

Inactivation, on the other hand, is associated with a decrease in Vmax for the same enzyme.

Differences in the sensitivity of \( \beta \)-adrenergic responses in the rat cerebral cortex cycles and after ovariection. H. Evian Wagner* and James N. Davis, (Spon: A. D. Roses), VA Medical Center and Duke University Medical Center, Durham, R. C.

We have previously reported a decrease in \( \beta \)-adrenergic recep­tor-mediated cyclic 3',5'-adenosine monophosphate (cAMP) response in the cerebral cortices of ovariectioned rats chronically exposed to 17α-ethynyl estradiol (E2) and 17α-ethynyl estradiol + 17β-estradiol (E2 + E2) for 10 days. Continuing with these studies, we now report differences between \( \beta \)-adrenergic receptor-mediated responses in the cerebral cortices of adult female rats exposed to the four different hormone regimens as well as between cycling females and adult male and ovariectioned female rats of the same age.

The results suggest, 1) that an amineric projection from the developmental homogeneate under phosphorylating or control (no cAMP or Mg-ATP) conditions. After a 3 minute pre-incubation following pre-incuba­tion of the homogeneate under phosphorylating or control (no cAMP or Mg-ATP) conditions, there is a 2-fold increase in enzyme activity (11.0 vs 5.7 molmes/hr control). Both phosphorylation activa­tion and inactivation are enhanced by the addition of electro­pheretically homogeneous bovine brain protein kinase catalytic subunit. The 6-MMP, Km for control, activated and inactivated enzyme are 0.86, 0.32 and 0.38 mM, respectively. Inactivation, therefore, is not due to decreased affinity of the enzyme for c cofactor. We also find that the Ki for dopamine is increased in both the phosphorylated activated and inactivated enzyme: control, 6.1 \( \mu \)M; activated, 37 \( \mu \)M; inactivated, 59 \( \mu \)M. Phosphorylation inactivation, on the other hand, is not due to an increase in affinity of the enzyme to an end-product feedback inhibitor. Phosphoryl­ation inactivation is associated with a decrease in the apparent Vmax. Additional experiments are required to determine whether phosphorylation inactivation results in a change of a particular characteristic in the physiological situation.

Concurrent with this work, we developed a new DOPA decarboxylase inhibitor: the 6-MMP. This compound, in a dose range of 0.1 to 1.0 mmol/l, inactivated DOPA decarboxylase in the presence of low voltage paper electrophoresis. This work was supported by USPHS Grant NS-11310.

Recently defontmity and Aghajanian reported (Science 202: 1265 (1978)) that 1-2 wk treatment of rats with tricyclic antidepressant drugs, there was a selective increase in the depressive response of hipocampal and lateral geniculate neurons to norepinephrine (NE). When iontophoretically applied on a 5-msec pulse at 3 Hz, the 5-HT (5-hydroxytryptamine) of the aim of the present study was to determine whether this finding held for amygdaloid (AMYG) cells. In addition, the effect of chronic, tricyclic antidepressants on the inhibition of AMYG cells by stimulation of the ascending 5-HT pathway was tested.

Drugs or saline were given ip for 2 wk. Chorlal hydrate anesthetized rats for single-unit recording and microiontophoresis. Glutamate (Glu) was used to activate cells so that both spontaneously active and quiescent neurons could be studied. To minimize variation in the firing level of cells, the maximum firing rate (prior to depolarization block) induced by ionto-Glu was determined for each cell. One half of the maximum firing rate was then maintained by adjusting the current of Glu. The sensitivity of AMYG neurons to 5-HT and other drugs was evaluated by the charge (the product of the current and the time) required to obtain a 50% decrease in the baseline firing rate.

The response of AMYG cells to 5-HT and lysergic acid diethylamide (LSD, a partial 5-HT agonist) was enhanced (1.5-3 fold) 24 hrs after 2-3 wk treatment with tricyclic antidepressant drugs (imipramine, desipramine or iprindrole). However, the increased responsiveness of AMYG neurons was not specific for 5-HT; the response of cells to norepinephrine (NE) was also enhanced. AMYG neurons were more sensitive to endogenous 5-HT released by stimulation of the 5-HT pathway near the dorsal raphe nucleus (DRN). Compared to controls, the threshold for 5-HT-induced depressant effects on AMYG cells was significantly decreased. Chronic treatment with chloropromazine, a tricyclic antipsychotic drug, failed to alter the responsiveness of AMYG neurons to 5-HT or NE.

The effect of chronic chlorpromazine treatment with tricyclic antidepressants, the enhanced receptor sensitivity began to disappear; in some cases, subsensitivity was observed.

The results of the present study show that chronic treatment of rats with tricyclic antidepressant drugs increase responsiveness of AMYG neurons to 5-HT and NE. It is suggested that modulation of the sensitivity of postsynaptic 5-HT and NE receptors may be related to the delayed therapeutic actions of tricyclic antidepressant drugs (Supported by USPHS Grants MH-17671 and NH-14459).

MONOAMINERGIC SYSTEMS

EFFECTS OF INTRAVENTRICULAR 5-HYDROXYTRYPTAMINE ADMINISTRATION ON THE ACQUISITION AND PERFORMANCE OF A SPECIFIC AND NOVEL LOCOMOTOR TASK IN RATS. Watson, W.,* and Mclellittat, J.B. (SPON: S. Mclellittat). Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Numerous investigators have proposed that catecholamines, notably norepinephrine (NE), are involved in learning processes. A method for eliciting the effect of central NE depletion on acquisition and performance of a specific task requiring precise paw placement has been developed. Water-deprived rats were trained to traverse an elevated arena placed at regular intervals (standard rod arrangement) for a water reward. Daily performance was assessed by determining average running time for approximately 25 s per day. A comparison of the spinal cord was then made. Antidromic activation was assumed when observing invariance of latency in response to 3 Hz. stimulation, the ability of a cell to fire 60 Hz. or more, or continuous firing. Antidromic activation was defined by antidromic activation via ball electrodes placed on the dorsal lateral surfaces of the spinal cord between T8 and T10. T10 was identified by antidromic activation via ball electrodes placed on the dorsal lateral surfaces of the spinal cord between T8 and T10.

The role of spinal cord activation on the basis of spinal cord velocity, cells in the 0.7-1.0 M/sec range were the most numerous. The units in the 3 to 6 M/sec range also declined significantly. In addition, antidromically activated cells in the NRM. On the basis of conduction velocities, antidromic activation was assumed if microiontophoretic application of putative transmitters, are modulatory effects on spinal cord transmission?

As reported previously, granule cells are systematically branched processes, whereas serotonin neurons and their processes develop early in prenatal ontogeny, the cerebellum develops late, postnatally. The aim of these experiments was to examine the effect of raphe transplants upon the subsequent development of the cerebellum in early postnatal rats. A series of experiments was conducted to determine the optimal ages of donor and host animals. Donor rats, 5-6 days of age and host animals of 4 to 14 days postnatal age were used. The most consistent results were obtained with both donor and host animals of 6 postnatal days. The midline raphe structures of the medulla were carefully dissected from brains of donor animals. Host animals were anesthetized with ether and the IVth ventricle exposed to receive the transplant without mechanical traumatizing the overlying cerebellum. Post-transplantation survival was 4 weeks and longer. The cerebellum and structures bounding the IVth ventricle were examined in one group of experimental animals following perfusion fixation with aldehydes and staining of the sections with thionin. Cerebellar structures close to the transplant consistently showed degeneration. The degeneration was observed early in postnatal life and persisted throughout the experiment. Any dorsal fornix malformation particular in the vermis, (2) foci of arrested granule cells due to nonextension of the external granular layer, (3) foci of disrupted histochimical terminals, the locus coeruleus and the subjacent subcoeruleus/parabrachial regions appear to provide differential descending noradrenergic innervation of cranial sensory, motor and visceromotor cell groups. (Supported by NS12481).

HISTOCHEMICAL AND HISTOFLOUORESCENT LOCALIZATION OF BIogenic AMINE DEPOSITS IN ALTERNATE SECTIONS. Joe Wood and Robert E. Mcclung. Dept. of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77025.

Biogenic amines can be readily detected in the CNS by histochemical methods, the techniques are simple, rapid and yield striking photographs; however, the reaction products are sensitive to light and tend to fade. Moreover, histofluorescent techniques are better. Low power light microscopy which reveals little of the non-fluorescent cytoarchitecture becomes unnecessary. Electron microscopic localization of biogenic amines using glutaraldehyde-dichromate has been developed. It is more sensitive than the histochemical system, and there have been light microscopic observations made from small plastic embedded sections. Difficulties of correlation of histofluorescence with light and electron microscopy are now more apparent than real. Fresh brain material can be frozen on dry ice and sections made on a cryostat at approximately 10-μm thickness. A tissue was fixed with glutaraldehyde, rinsed, and sectioned on a cryostat. Sequential sections were alternated, with section A being used for histofluorescence, section B for a modified glutaraldehyde-dichromate procedure, and section C as an untreated control. Glyoxylic acid histofluorescence shows norepinephrine and serotonin-containing cell bodies and processes, especially in the brain region.

The next section was treated with a mixture of glutaraldehyde, sodium chromate and potassium dichromate at pH 4.1. Following incubation the tissue was stained with conventional histologic stains, i.e. cresyl violet and/or toluidine blue. Neurons correlative with histofluorescent areas were seen to contain catecholamine and/or indolamine chromine deposits in cell bodies and in some processes. Visualization of these deposits range anywhere from low-power light microscopy to oil immersion, and with care, pigments can be differentiated from amine deposits. In the sustained NE treated control section, only pigment deposits are visible. This technique should lead to a better understanding of the cytoarchitecture of the central nervous system and the neuronal-chemical connections within the brain cells, i.e. glial cells and other neurons. The technique will be useful in localizing amine-positive areas for electron microscopy, and no doubt will serve as the bridge from mapping to electron microscopy. Supported by USPHS grant NS-10326.

COMPARISON OF NORADRENERGIC MODULATORY ACTIONS ON PURKINJE CELL RESPONSES TO IONTOPHORESIS OF γ-AMINOBUTYRIC ACID, 5-BALANINE AND TAURINE. Hermes H. Yeh, Dylan C. Nokes, Barry D. Waterhouse and Donald J. Woodward. Dept. Cell Biology, U. Texas Health Science Center, Dallas, TX 75235.

We have previously shown that both iontophoretically applied norepinephrine (NE) and stimulation of the locus coeruleus can potentiate γ-aminobutyric acid (GABA)-mediated inhibition of cerebellar Purkinje cells (PC). Histological studies do not establish NE and dopamine did not mimic the facilitatory effect of NE. The specificity of the observed NE modulation was studied further in a series of pharmacological experiments comparing depressant amino acids structurally related to GABA.

Multibarrel micropipettes were used to apply drugs and record extracellular PC responses in halothane-anesthetized rats. The gabaergic receptors are not dependent on a specific amino acid, but are sensitive to a wide range of amino acid compounds, neurotransmitters and drugs. PC responses to superimposed NE pulses (10 sec duration at 30-35 sec intervals) of β-alanine, taurine and GABA were examined before, during and after NE iontophoresis (5-20 na). Drug response histograms were computed to compare NE effects on spontaneous and drug-induced neuronal activity.

Consistent with previous findings, NE at low or no effect on PC spontaneous activity produced marked augmentation in all 13 cells tested. In 5 cells, GABA applied continuously at low currents showed no effect on PC responses to superimposed GABA pulses. This suggests that the observed modulation with NE cannot be explained by summation of NE and depressant effect of NE. Comparison of NE effects with those of NE interaction with β-alanine-induced inhibition, tested in 19 PC's, was clearly different from GABA, with a predominance of 10 cells showing no effect, 2 cells showing NT greater than control, and NE effects on taurine-induced inhibition was also dissimilar to GABA. In 14 cells tested, 6 showed no significant changes, 4 cells were potentiated and 4 cells depressed. In 2 cells, NT and NE appeared to have antagonistic effects, when seen with β-alanine and taurine, were moderate in comparison to that of GABA. Picrotoxin reversibly blocked GABA (5 cells) and β-alanine (3 cells) induced NE potentiation, NE effects appeared to be distinctly longer duration. This preliminary result, in addition to the apparent specificity in NE modulation, may argue for a role of the NE receptor in regulating different amino acid receptors or, perhaps, reuptake processes.

In summary, these data confirm the previously reported NE modulation of GABA responses and add to the evidence that NE preferentially enhances the inhibition of PC's produced by iontophoretically applied GABA in contrast to that produced by other amino acid neurotransmitters. Support for this research was supported by grants from NSF BNS77-01174, NIDA DA-02338 and the Biological Humanics Foundation to DJW.

MONOAMINERGIC SYSTEMS
ELECTROPHYSIOLOGICAL EVIDENCE OF INHIBITORY INPUTS TO THE VENTRAL TEGMENTAL AREA FROM THE PREOPTIC AREA AND THE NUCLEUS ACCUMBENS.


Previous anatomical studies have shown that limbic forebrain structures project to ventral tegmental area (VTA) of the mid-brain (Nauta, Brain 1958, 81, 319). The present study investigated with electrophysiological recording techniques possible inputs to the VTA from the anterior hypothalamic-medial preoptic area (AHPO) as well as from the nucleus accumbens (NA).

Action potentials were recorded extracellularly from neurons in the VTA of urethane anesthetized rats using glass micropipettes filled with 4M NaCl. The effects of single pulse stimuli delivered to the NA and AHPO on the neuronal activity of the ipsilateral VTA were investigated. In some experiments, 7 barrel micropipettes were used for recording and iontophoretic application of picrotoxin (a GABA antagonist) and nipecotic acid (a GABA uptake inhibitor) to study the effect of these drugs on the response of VTA neurons to NA stimulation.

The electrophysiological characteristics of the population of units recorded suggested two types of VTA neurons: one type had long spike durations and slow rates of discharge and a second type shorter spike durations and fast rates of discharge. These observations confirm previous findings in which the two types were considered to be dopaminergic mesolimbic and non-dopaminergic neurons (Yim & Mogenson, Brain Research 1979, in press). Approximately 20% of VTA neurons were antidromically activated from the nucleus accumbens.

Both types of VTA neurons received convergent inputs from the AHPO and NA. Electrical stimulation of the AHPO inhibited the majority of the sample of VTA neurons tested with a relatively short latency (<10 ms). Stimulation of NA inhibited both types of VTA neurons tested but in addition, activated the first type of VTA neurons as well. Activations usually had longer latencies of >10 ms. Inhibition of the VTA neurons by stimulation of NA was blocked by iontophoretically applied picrotoxin and prolonged by nipecotic acid with no change in latency.

These observations provided evidence of an inhibitory input from AHPO and NA of the limbic forebrain to dopaminergic and non-dopaminergic neurons in the VTA. The effects of the iontophoresis of picrotoxin and nipecotic acid suggest that the descending inhibitory pathway from the NA to the VTA is GABAergic. Nature of the transmitters that mediated the excitatory response from NA and the inhibitory response from AHPO stimulations remains unknown. The descending GABAergic projection from the NA to the VTA appeared to be similar to the feedback GABAergic pathway present in the nigral striatal dopaminergic system.

(Supported by MRC of Canada)
MOTOR SYSTEMS
1201 THE NATURE AND DISTRIBUTION OF NECK MUSCLE AFFERENTS PROJECTING TO THE MEDULLA. V.C. Alabans and T. Yokota, Department of Physiology, Queen's University, Kingstone, Ont. Canada, K7L 3N6.

Electrical stimulation of muscle afferent nerves provides a convenient technique for studying central projection pathways. Afferent nerves were stimulated by a large dorsal root ganglion, and the antecedent activity was recorded from EEG electrodes as a function of stimulation frequency. The afferent fibres activated were mostly between 4 and 8 msec. The same units responded to mechanical stimulation with large nociceptive cutaneous fibres or at higher strengths from 2 to 10T will lead mainly to excitation of spinules and GTO's. This stimulation of the large dorsal neck muscle afferent nerves at strengths of 3 to 2T will lead mainly to excitation of spinules and GTO's. This is only when stimulus strengths from 2 to 10T are used that GTOII afferent fibres are activated. Stimuli of 10T and greater (and usually requiring longer pulse widths) are necessary for unmyelinated afferents to be excited.

These stimulation criteria were used to re-examine the nature of the afferent fibres of the neck muscles and to determine the distribution of this activity has been compared with the MUA distribution of this activity has been compared with the MUA of muscle afferent projections to the medulla just posterior to the obex. Fast green dye-filled glass micro-electrodes were used so that recording sites could be accurately noted. The largest group of units activated by neck muscle afferents and few spinule or GTO projections were found and then in widely scattered regions. The largest population of units excited (4 of 38 examined) had locomotor afferent fibres and 36 units were below 4T. The same units responded to mechanical stimulation of the ipsilateral corneal (usually light touch of cotton, but in 3 units) pinch of the pinch pinnae (4 units) and to a range of low to high thresholds (2.5 to 2T) and with large nociceptive cutaneous fields were found ventromedially to paraganglionarions of trigeminal subnucleus caudalis. Low threshold units were found as previously described in the most lateral region of the cuneate nucleus and between the cuneate nucleus and trigeminal subnucleus caudalis. Supported by M.R.C. of Canada.


Vibration applied to the tendon of the extensor muscles of the ankle joint produces a myotatic reflex. This is in contrast to the facilitation of the myotatic reflex produced by tonic voluntary contraction.

Vibration does not influence the post-myotatic component of the stretch evoked response. This component is also not significantly influenced by the tonic voluntary contraction. These results indicate that the post-myotatic responses to limb perturbation are not only different from the myotatic reflex in their latency but also in their functional dependence to peripheral and central influences.

The mechanical effects of vibration were measured by applying sinusoidal or random torque inputs. Although the myotatic response to discrete stretches is suppressed by the amplitude and frequency of sinusoidal oscillation that produce an average EMG response which is different in magnitude from the no-vibration condition. The stretch responses of sinusoidal oscillation produce an average EMG response which is not different in magnitude from the no-vibration condition. The stretch responses of sinusoidal oscillation produce an average EMG response which is different in magnitude from the no-vibration condition. The stretch responses of sinusoidal oscillation produce an average EMG response which is different in magnitude from the no-vibration condition. The stretch responses of sinusoidal oscillation produce an average EMG response which is different in magnitude from the no-vibration condition.


The firing patterns of cortical neurons associated with conditioned hand movements have been extensively studied in the monkey using single unit techniques. These studies are usually limited to recordings from a restricted region and are inherently biased toward sampling of large neurons. In this investigation, we have examined digitally rectified and averaged multiple unit activity (MUA) recorded simultaneously from the frontal and parietal cortex of monkeys trained to perform self-initiated hand movements. The timing and intracortical distribution of this activity has been compared with the MUA elicited by peripheral stimulation. Our recording methods provide a weighted sum of the neural firing within a sphere of tissue roughly 350 μ in diameter, and thus, provide an index of net changes in neural population activity within a circumscribed cortical locus. These data are particularly useful in interpreting field potential recordings, which provide the only available method for studying human sensorimotor processes.

Antecedent increases in MUA begin 90-100 msec before EMG monitored contractions and are recorded throughout the hand area of the precentral gyrus, including the anterior bank of the central sulcus. The antecedent activity is maximum in amplitude within the fifth lamina but can also be recorded in the fourth and fifth lamina. In the more superficial laminae of area 4, the initial increase in MUA is coincident with movement onset and continues for up to 200 msec following the end of EMG activity. Peripheral stimulation of the median nerve which is sufficient to produce a thumb twitch in activation limited to the posterior portion of area 4. Within area 3a, a phasic burst of MUA begins 10-20 msec after movement and is followed by a second discharge which peaks 100 msec after movement onset. Area 3a receives the earliest somatotopically input with MUA beginning 6.0 msec after stimulation with median nerve. MUA in area 1 and 2 follows the onset of contraction and also peaks at 100 msec. Additional MUA which begins 150 msec after movement onset is recorded from area 5. Movement onset in MUA is found in area 7 associated with self-paced movements. This work was supported by Grant NIH 6723 from the USPHS.

The effect of gamma fusimotor innervation on the transducing properties of the muscle spindle receptor were studied in the tenuissimus muscle of the cat. Functionally single primary afferent axons and static gamma motor axons were isolated from dorsal and ventral rootlets at the L7-S1 level. Length perturbations having a Gaussian amplitude distribution of 10-100μm RMS level and a flat frequency spectrum from DC to 120 Hz were applied to the proximal half of the muscle and the lA discharge recorded, with and without concurrent, independent Poisson pulse trains of 40-80 pulses/sec mean rate being delivered to one or more gamma axons. Crosscorrelation of the Ia spike train with the input length waveform allowed the calculation of the average prespike length of the impulse response function at each frequency were observed to accompany the gamma function provided an estimate of the receptor's frequency response. 

In the absence of gamma stimulation the gain of the frequency response curve was increased at high frequencies, suggestive of a system responding to the velocity of the applied perturbation. 

When individual gamma axons were stimulated the relation was shifted by varying amounts for each axon, towards 400 decade, a characteristic of an acceleration-sensitive system. When pairs of gamma axons were independently stimulated, the shift was generally greater than that seen from a single axon, but was never greater than 400 decade even for cases where one of the axons produced such a shift when stimulated alone. 

In mean spike rate and an absolute decrease in the magnitude of the gain function at each frequency were observed to accompany the gamma effect on the slope of the gain curve (Supported in part by NIH grants NS 11295 & GM 23732).

1206 THE SIZE PRINCIPLE: EFFECT OF INHIBITORY INPUTS TO MOTORNEURONES IN HUMAN SUBJECTS. P. Bane, Dept. of Kinesiology, S.F.U., Burnaby, B.C., VSA 150.

In the decerebrate cat, Henneman and coworkers (1965, 1974) demonstrated that the effect of an inhibitory input to a motoneurone pool with an existing excitatory input is to reduce the effect of excitation, thus keeping the fixed order of recruitment of motoneurons. The following experiments were done to test the effects of inhibition in normal human subjects. The subject sat comfortably in a chair with his foot strapped to a plate carrying strain gauges that the contractions of T.A and triceps surae (G-S) muscles were isometric.

(i) Dsynaptic spinal inhibition: The subject recruited a pair of distinct single motor units (SMUs) from T.A and maintained their firing rate. Stimulus to the tibial nerve to elicit H-reflex in soleus resulted in inhibition of T.A motor units. The high threshold (HT) SMU dropped out before the low threshold (LT) SMU for an interval up to 300 msec. No reversal in depennent observed.

(ii) Vibration of Achilles tendon: The subject recruited two distinct SMUs from T.A and maintained their firing rate. Vibration of Achilles tendon at 60Hz, resulted in TVR in soleus and inhibition of T.A-SMUs. The HT-SMU decreased before LT-SMU without exception.

(iii) Descending inhibition: Achilles tendon was vibrated at 60Hz to recruit two distinct SMUs from soleus. 

The subject was asked to depress these units voluntarily while the vibration was still on. Out of the 50 pairs studied, 34 decreased with HT-SMU before LT-SMU. In 12 pairs, LT unit dropped before HT unit while 5 pairs were "confused" the order of recruitment & decrement changed on rechecking.

The net excitability of a motoneurone is determined by the temporal and spatial relationships of excitatory and inhibitory synaptic inputs. In the first two cases, if there is only one inhibitory input which has a fixed spatial relationship to the excitatory input, a uniform reduction in the excitatory conductance change would result in a regular pattern of decrement. In the last case, if the additional inhibitory conductance change is from G.T.O.s (60Hz recruits G.T.O.s), Renshaw cells and PAD could interact in a more complicated way to result in the observed pattern of decrement. This implies that conductance changes are not the only determinants of decrement, the interaction of IPSPs versus EPSPs have to be taken into account. This work was supported by Pres. Res. Grant at S.F.U. and B.C.H.C.R.F.


The sensitivity of the vestibular otolith organs is lowered whenever the head is tipped out of its normal orientation. Recovery (Melvill Jones & Young, 1979) is due to an increase in the threshold of sensitivity to saccular stimulation in the otolith-vestibular reflex. (Supported by NIH grants NS 11295 & GM 23732).

The sensitivity of the vestibular otolith organs is lowered whenever the head is tipped out of its normal orientation. Recovery (Melvill Jones & Young, 1979) is due to an increase in the threshold of sensitivity to saccular stimulation in the otolith-vestibular reflex. This movement deficit has variously been attributed to learned non-use, cross-spinal inhibition, sensory inattention, or, most parsimoniously, preference for use of the fully innervated limb. This study indicates a further possibility, that dorsal column and dorsal column nuclei are implicated in the processes underlying loss of function after dorsal rhizotomy.

Eight monkeys underwent dorsal rhizotomy C2-T3 (DR) and/or dorsal column (DC) section and/or dorsal column nuclei (DCN) lesion. The time between procedures was four to six months. The lesions were: in three monkeys, bilateral DCN lesions and DC section by unilateral DR; in two, bilateral DCN followed by unilateral DR; in two, unilateral DCN lesion followed by bilateral DR; and, in one, unilateral DC section followed by bilateral DR. Animals were maintained in a 14 x 9 x 8 cage with wire mesh walls and narrow beams affording opportunities for ambulating and climbing.

In every case in which DR was combined with DCN lesion or DC section, use of the DR limb was seen from the first postoperative day. This was true when DCN lesion or DC section preceded DR and was also the case when DCN lesion preceded DR. The limbs of these monkeys were used for support, climbing, ambulating, reaching for food placed on the floor or hanging from the ceiling, and for bimanual tasks. The animals with bilateral DR used combined lesion limbs to the exclusion of contralateral DR-only limbs. The limb with both DR and DCN lesion was also often preferred over the limb with DCN lesion-only for performance of tasks requiring fine digital coordination. This presumably was due to the poor coordination of fingers in the hand ipsilateral to DCN lesion. This loss of coordination was significantly alleviated by superimposed dorsal rhizotomy.

It would appear that, after DR, the dorsal column, dorsal column nuclei system exerts inhibitory effects on movement. Lesions of these pathways remove this inhibition, allowing return of motor function.

Mechanisms underlying head movements elicited by activation of the superior colliculus have been investigated by recording activity of neck muscles and reticulospinal neurons in pretectal decerebrate cats during microstimulation of the intermediate to deep tectal layers. Trains (duration 30–400 msec, rate 300–400 Hz) of 100 usec pulses with amplitude >0.100 VA produced rapid, evanescent eye movements and excitation of contralateral dorsal and ventral neck muscles plus inhibition of corresponding ipsilateral muscles. Excitatory RST neurons in these muscles typified that of an initial burst at a latency of 5–13 msec (mean 9 msec) followed by rebound depression and then by tonic excitation which maintained the movement in the train. The trains also produced excitation of postural anterior muscles. Muscles with upward pulling directions (rectus major, biventer, complexus) tended to contralaterally evoke discharge of 2.5 to 10/sec sec with a median of 5/sec. Some neurons were activated for the entire duration of the stimulus train, others responded only at train onset or exhibited a combination of the two patterns. Discharge of these neurons is therefore appropriate to facilitate the responses of neck muscles to tectal stimulation. Such neurons were most prevalent among RST neurons located in the intermediate tectum. These neurons may participate in vestibulo-neck reflexes stimulation of contralateral semicircular canal nerves. contribution to the responses of neck muscles to tectal stimulation. Such neurons were most prevalent among RST neurons located in the intermediate tectum. These neurons may participate in vestibulo-neck reflexes stimulation or the release of the force transducer from its grasp with an apparatus. Immediate following ablation of the SMA a significant increase in the rectified EMG activity of the forearm muscles was observed at 25 minutes. A comparison of the 10 and 20 gram sustained force application showed a 50% decrease in CAP occurring at 2 minutes for the 20 gram weight and 60 minutes for the 10 gram weight. The initial post-ablation depression decreased in amplitude as a function of compressive force, indicating a decrease in the population of larger diameter fibers. The other phases of the CAP remain relatively unchanged throughout the course of nerve regeneration. Cause injury requires that nerves be manipulated with care. Muscle force appears primarily to affect the structural integrity of the larger fibers. The rate and extent of the damage is dependent on the magnitude and duration of the compressive forces. The relatively low magnitude of the forces that cause injury requires that nerves be manipulated with care. These effects are more pronounced in a severed nerve. (Supported in part by Liberty Mutual Insurance Company)

DISTURBANCE OF BOTH CONTROLLED PREHENSION AND GRASP RELEASE FOLLOWING UNILATERAL ABLATION OF THE SUPPLEMENTARY MOTOR AREA. Daniel Bourdouans*, Allan M. Smith and Gilles Blanchette*. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec.

The effects of unilateral supplementary motor area (SMA) lesions on the performance of a learned precision grip of the contralateral hand was studied in fascicularis monkeys. Animals were first trained to exert an isometric force between the thumb and forefinger for a one-second duration to obtain a fruit juice reward. Finger pressures were measured by a hand-held strain gauge and a tone was given that the pressures were within an upper and a lower force limit. On both rewarded and unrewarded trials the animals were required to release the grasp on the transducer in order to initiate the next trial. Pre-operative surface recordings from the forelimb flexor and extensor muscles of the wrist and fingers indicated that the maintained prehension was accomplished by a co-contractile force activity. Immediately following ablation of the SMA a grasp reflex appeared in the contralateral hand which persisted for 2 to 3 weeks. Grasping was easily elicited by cutaneous stimulation of the volar surface of the hand and could be augmented by stretch of the finger muscles. The most striking disturbance of the conditioned precision grip was the inability to release the force transducer from its grasp with the contralateral hand. In contrast, release from the ipsilateral hand remained normal. A conditioned prehension produced a significant increase in the rectified EMG activity of the forearm flexor muscles and a significant decrease in the forearm extensor muscles when compared to the pre-operative period. The corresponding increase in the rate at which prehensile force was applied to the transducer developed during the three week post-operative observation period. Moreover, the median finger pressures exerted by the hand were greater than the mean pre-operative levels. Repeated elicitation could habituate the grasp reflex and allow the monkeys to release the effects of the contralateral grip under voluntary control. Frequent, release of the manipulandum was preceded by a sudden violent increase in prehensile force as the animals randomly closed their grasp voluntarily. Opposite the hand either by habituation of the grasp reflex or by the facilitation of opposing reflexes. The extent of the cortical lesions in both monkeys has been histologically controlled by serial section. This research was supported by a Medical Research Council of Canada grant to the group in Neurological Sciences at the University of Montreal.
1212 EFFECT OF HEMISECTION ON MOTOR DEVELOPMENT IN KITTEN HINDLIMB. Barbara S. Bregman and Michael E. Goldberg. The Medical College of Pennsylvania, Philadelphia, PA 19129.

At birth, immature proprioceptive placing is present in all directions, but is unreliable, high threshold and hypermetric. By 2 weeks, it is abnormally delayed and afferent input is seen at 22-23 days but is immature until 6 weeks when it is smooth, accurate and of small excursion. Monopodal hopping is present by 2 weeks and is bilateral. Tactile evoked reflexes are seen at 22-23 days but are immature until 6 weeks when they are smooth, accurate and of small excursion. Monopodal hopping is present by 2 weeks and is bilateral. Tactile evoked reflexes are seen at 22-23 days but are immature until 6 weeks when they are smooth, accurate and of small excursion. Monopodal hopping is present by 2 weeks and is bilateral. Tactile evoked reflexes are seen at 22-23 days but are immature until 6 weeks when they are smooth, accurate and of small excursion.

In the hindlimb, afferent activity is seen in single DR VN (n=4) and GG area (n=2) conditioning was seen. Suprathreshold in L6-S1 spinal cord, posterior to the lumbar enlargement, vestibular nuclei (VN) (n=15) and GG area (n=20) facilitated 50 shocks, 200 μA applied to VMH never facilitated ipsilateral stable segmental responses were obtained, similar facilitation by shocks. Conditioning by RF (requiring 1-5 shocks) appeared with short-latency responses were more consistently obtained for ML and areas lateral and ventral (CG area), and ventromedial hypotautism were transiently impaired and then appear to recover. As the reaction is delayed 3-6 days but its maturation is not delayed. During the first 3-4 weeks the hemisected kittens show a slippage deficit in proximal fixation ipsilaterally. Then the kitten grows into some deficits, i.e. they don’t appear until the 2nd month. They do not recover.

Ipsilaterally, tactile placing is essentially abolished, although occasionally a gross placing response to tactile stimulus is elicited. Proprioceptive placing and hopping which had appeared to recover become normal (high threshold, hypermetric).

A rise of the hindlimb muscles in the hemisected animal, locomotor deficits in the hemisected animal appear. Ipsilaterally, the dorsum of the foot always draws at the initiation of swing phase. The limb flexes hypermetrically and at the hip and becomes even more severe. Reflex and locomotor development are integrated in both normal and hemisected kittens. Some reflexes are transiently impaired and then appear to recover. As the reflex normally becomes more dependent on higher control, recovery compensates. Some reflexes and locomotor patterns are initially dependent on supraspinal control; they normally appear late in development. Hemisection arrests the development of these reflexes and locomotion reveals permanent immaturities.

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1214 SUPRASPINAL AND SEGMENTAL INFLUENCE ON MEDIAL AND LATERAL LONGISSIMUS NERVE ACTIVITY IN RATS. Emily Brink* and Donald W. Pfaff. The Rockefeller University, New York, NY.

Medial longissimus (ML) is a proximal tail muscle, innervated by L4-L5 nerve branches. Lateral longissimus (LL) is a lumbar back muscle, innervated by dorsal rami along its length. Inputs to these muscles were studied in urethane-anesthetized female rats by recording from ML and LL nerves while stimulating ipsilateral lumbosacral dorsal roots (DR), medullary medullary reticular formation (VMH), vestibular nuclei complex (VN), midbrain central gray (CG) area, and ventromedial hypothalamus (VMH). Stimulation of appropriate DR evoked short-latency (probably polysynaptic) compound potentials in ML (1.8-2.2 msec, n=40) or in LL (2.1 msec, n=14) nerves. Stable short-latency responses were more consistently obtained for ML (40/50 cases) than LL (10/17 cases). Generally, ML responses required double shock (1.2-2 msec interval); LL response required 3-4 shocks. Supraspinal influence was studied using a condition-test paradigm. Subthreshold pulse trains of variable length (cathodal pulses, -1 msec duration, 100 μA, monopolar) preceded subthreshold DR single shocks. For ML nerves, conditioning stimulation of RF (n=14), VN (n=15) or CG area (n=20) facilitated occurrence of short-latency segmental responses to single DR shocks. Conditioning by RF (requiring 1-5 shocks) appeared with condition-test intervals (CTi) of 0.5-2 msec. VN conditioning (CS) reduced CTi 2-30 msec. Conditioning by CG (9-20 shocks); optimum CTi 5-20 msec. In the lesser ML nerves where stable segmental responses were obtained, similar facilitation by VN (n=12), and CG area (n=22). Conditioning of RF, VN or CG area stimulation could provoke measurable potentials in ML (n=31,2) and LL (n=1,4) nerves. Stimulus trains of up to 50 shocks, 200 μA applied to VN motor neurons produced conditioning to ML or LL nerve activity. Results indicate that excitatory pathways from RF, VN, CG area to ML and LL exist and suggest some differences in spinal modulation of these nerves.

ML motoneurons localized by antidromic stimulation were found in L4-S3 spinal cord, posterior to the lumbar enlargement, ventrally laterally in the medulla were found medially in the ventral horn of the lumbar enlargement.


A triphasic pattern of EMG activity has long been recognized in association with voluntary movements. This pattern consists of an initial burst of agonist activity followed sequentially by a burst of antagonist activity in the antagonist and a final period of agonist activity. The experiments described here were designed to investigate the interaction between this common pattern of EM activity and imposed perturbations in limb position. Experiments were performed on normal human subjects performing a visual step-tracking task using flexion/extension movements about the elbow. Torque pulse perturbations were applied randomly during trials and were timed to occur following the change in target position but before the onset of movement. Perturbations both opposing and assisting flexion were applied (flexion load and flexion unload forces).

The response to the force opposing movement appeared as a second peak of biceps burst in the latter phase of the first biceps burst. This peak was present in some subjects in the absence of perturbation. Changes in delay time of the perturbation relative to movement onset did not alter the latency of the response. Little change was seen in the other movement-related EM activity except for an increase in the amplitude of the triceps burst. No EMG responses were seen corresponding to the ML or VL flexion responses. These flexion responses were, however, present when perturbations were applied following movement onset or when the subject was required to perform a fixed position. Application of perturbations antagonizing movement (flexion unload) produced a decrease in this later component of the biceps burst. The results suggest that for some period prior to movement onset, reflex systems and the systems generating the initial burst of agonist activity are inaccessible to peripheral modification.

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1216 EFFECTS OF VIBRATORY MUSCLE STIMULI APPLIED DURING VOLUNTARY HUMAN ARM MOVEMENT. C. Casadio* and J. D. Cooke, Dept. of Physiology, Univ. of Western Ontario, London, Canada.

Vibration of muscle tendons has been used by several investigators to study the "passive sense of position". In the present study, the effects of such vibratory muscle stimulation on active arm movements has been investigated. Subjects performed a step-tracking task making alternate flexion/extension movements about the elbow. They were instructed to move as accurately and precisely as possible between the target zones. All subjects were well practiced at the task. No differences were seen in movement trajectories if the subjects performed the task with or without visual guidance.

A small DC eccentric motor enclosed in a plastic casing (weight approx 70 gm) was mounted on either the biceps or triceps tendon using adjustable Velcro straps. During continuous vibration of either tendon the amplitude and peak velocity of both flexion and extension movements were decreased. The changes were more noticeable in trials where the movements were performed without visual guidance (eyes closed). Similar changes in movement amplitude and velocity were seen when the vibration was applied just during the movements. Activity in reflex pathways was tested by application of torque pulse perturbations while the subject was required to maintain a fixed arm position. During vibration, either of tendon, short and long-latency reflex responses to the perturbation were markedly depressed in both biceps and triceps muscles.

The above results are consistent with activation of la muscle afferents by the vibration and with the results obtained from 'passive position sense' experiments. The results suggest that the effects of vibratory muscle stimulation on the parameters of active movement are produced by an action of muscle afferent input on supra-spinal structures.

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1217 SPINAL PROJECTIONS FROM THE MEDULLA OF THE ALBINO RAT. A.J. Castiglioni, Jr. and J. D. Coulter. Marine Biomedical Institute, Departments of Psychiatry & Behavioral Sciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

A mixture of Fast Blue (0.5-1.0 μl, 40% solution) was injected into various levels of the spinal cord of adult albino rats. The brains were cut into 50μm sections and the neurons containing Fast Blue were visualized with enhanced light microscopy (Hardy-Heimer method). At the level of the pyramidal decussation retrogradely labeled neurons were found in a dense band arching from the region dorsomedial to the lateral reticular nucleus toward the central canal ipsilaterally, and contralaterally in small numbers in the ventral reticular formation (R.P.F.) following injections of the thoracic cord. The lumbar cord produced many labeled cells predominantly medially and dorsally in the R.P.F., while lumbar injections yielded small numbers of labeled neurons in the ventral reticular formation. In the medullary R.F. at levels of the inferior olive, lumbar injections produced large numbers of labeled cells in the ventral R.P.F. overlying the inferior olive contralaterally to injections. Cells labeled from the thoracic cord were found lateral-dorsal to lumbar projecting cells, and cells labeled from cervical injections were medial-dorsal in the R.P.F. contralaterally to injections.

Injections in all spinal levels labeled cells throughout the medial reticular formation ipsilaterally at these medullary levels. Small cells in the marginal layer of the spinal nucleus of V were labeled in caudal portions of the nucleus from injections of the upper cervical cord (C5-C7). Rosintra many large neurons in deeper regions of the nucleus were seen after either upper or lower cervical levels. Cervical enlargement. Many small labeled neurons were found just ventral to the lateral tip of the inferior olive following intramedullar injections as well as labeled cells lying among fibers of the pyramid. At caudal levels of the inferior olive many labeled cells were seen in the nuclei of the raphé and in lumbar injection sites. Similarly, cervical levels many small cells are seen ventrally in the raphe, between the inferior olives, but relatively few were seen dorsally. A similar pattern is seen at both thoracic and cervical levels, however, the number of labeled cells is reduced. Many cells of the nucleus parvocellularis of the medullary R.P.F. were labeled after cervical injections, mainly in large numbers of cells in this region were labeled from injections into lumbar levels.

These results indicate that spinal projections from medullary reticular structures are less diffusely organized than previously thought. (Supported by NS12481).


In a previous communication (Chandler et al., Neuroscience abstracts, p292, 1978.) we have shown that the antidromic field potential recorded in the motor nucleus of the trigeminal nerve is depressed during active sleep as compared to quiet sleep. Additionally, we demonstrated that intracellularly recorded antidromic spike potentials in masseter nerve muscle were specifically blocked during the transition from quiet to active sleep. This was correlated with the onset of muscular atonia and membrane hyperpolarization. In this report we will present data indicating that these events are due to a process of postsynaptic inhibition of trigeminal jaw closer motoneurons during active sleep.

Intracellular recording from trigeminal jaw closer motoneurons with 2M K citrate micropipettes (8-15mΩ) during quiet and active sleep states produced by an action of muscle afferent input on supra-spinal structures.

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1219 SUBTHRESHOLD MEMBRANE ACTIVITY IN SPINAL CORD MOTONEURONS DURING ACTIVE SLEEP. Michael H. Chase and Francisco R. Morales*, Departments of Physiology and Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

During active sleep there is a decrease in the tone of the somatic musculature which occurs in conjunction with hyperpolarization of the membrane of α-motoneurons. Also, during this state, there are subthreshold membrane potential changes which may provide the substrate for the phenomenon of "passive sense". In the present study the above results are consistent with activation of la muscle afferents by the vibration and with the results obtained from 'passive position sense' experiments. The results suggest that the effects of vibratory muscle stimulation on the parameters of active movement are produced by an action of muscle afferent input on supra-spinal structures.

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Use of an external solenoid coil to apply force to a slug chronically implanted in the interpeduncular nucleus of Rhesus monkey (Colburn & Wolpaw, 1977). The coil produces force on the slug through the use of an iron sleeve that greatly reduces heating. Our coil measures 50 mm long, 42 mm o.f. the coil (Fig.). C oil inside diameter should be as small as practically possible, to maximize both force and frequency response. AC current can be used to deliver vibratory forces, but should be rectified to reduce power consumption. An iron sleeve around the coil gives a 50% increase in force. Water cooling by means of copper tubing wrapped around the sleeve greatly reduces heating. Our coil measures 50 mm long, 42 mm o.f. the coil (Fig.). C oil inside diameter should be as small as practically possible, to maximize both force and frequency response. AC current can be used to deliver vibratory forces, but should be rectified to reduce power consumption. An iron sleeve around the coil gives a 50% increase in force. Water cooling by means of copper tubing wrapped around the sleeve greatly reduces heating.


Subcortical projection sites from motor cortex have been identified in many animals using electrophysiological techniques. The quantitative subcortical distribution of different projections among all the different terminal fields has not been established. As part of an ongoing study of motor pathways during focal seizures it became important to know the site and relative density of first order projections from motor cortex. 


Ablation of either the superior colliculi (SC) or the frontal eye fields (FEF) produces relatively small deficits on eye movements. In both of these structures, however, single units discharge in association with saccadic eye movements and stimulation triggers saccades at low current levels and with short latencies. Recently it has also been shown that ablation of the SC does not interfere with stimulation elicited saccades from the FEF (Schiller, P. H. Brain Res. 122:154, 1977). This finding suggests that two structures form a parallel pathway to the motor cortex. Over 50% of the total amount of subcortical projections ended in Va (26.5%) - VL (30.1%) complexes. Ipsilateral (26.1%) and contralateral (24.5%) caudate/putamen terminal field densities occurred in a bi-laminar pattern in dorsolateral quadrants. The central medial (CM) (1.5%), and the central lateral (CL) 8.5%. 1-2% of the total was found in reticularis, zona incerta, pretectal, and the posterior nuclear group each. Less than 0.5% appeared in lateral medullary (LM), hypothalamus (H), and the parafascicularis n. and the peri-rubral field each. In addition to caudate there were very faint projections across midline in CL and VM.


The stretch reflex response of the soleus (SOL) (n=4) and medial gastrocnemius (MG) (n=18) muscles was observed by recording tension and EMG from whole muscle and single motor units during imposed 5 mm/sec stretches. Whole muscle EMG was recorded intramuscularly, fully rectified, and averaged. The EMG was also intramuscularly recorded, with fine-wire bipolar electrodes. During stretch responses, muscle force increased linearly while total electromyographic activity and recruitment remained essentially constant. We observed a rapid divergence of the force-EMG relations of isometric from that of lengthening muscle, indicating a trans­ient reduction in force production which occurred only after stretch onset. This increase in activation is generated by reflexively induced motor unit recruitment and rate modulation.

Recruitment is one of the obvious mechanisms for increasing force in numbers of newly recruited units with increasing force appear to be preserved in dynamically lengthening muscle. Force thresholds of motor units are increased in lengthening, compared to isometric muscle suggesting an overall broadening of the force range of the recruitment mechanism. In dynamically lengthening muscle, recruitment was observed throughout the force range obtained with MG stretch reflexes and at forces above 1 kg in SOL. 

Rate modulation was observed to occur with three basic patterns during stretch reflex activation: 1) step increases in rate just after stretch initiation, 2) doublets, in units activated both prior to and during stretch and 3) smooth increases in firing rate in units not recruited during the stretch. The smooth increase in rate observed for previously active units was 3.7±1.8 pps/100 g increase in force. Force thresholds of MG units were lower than those of SOL units.

Although the MG produced a smaller dynamic range of recruitment and rate modulation than MG, it appears to be significantly greater than that previously reported (1). Additional EMG was observed with MG stretch reflexes, which may be important for future studies. The proposed mechanisms is thought to represent reflex compensation for intrinsic non-linear muscle properties. The most prominent compensatory mechanism is likely to be one of the proposed mechanisms reported by previously reported electrical stimulation studies (2).


Force vs distance from middle of coil for a soft iron (Armco) slug and a permanently magnetized (Alnico) slug.

APPLICATIONS.
THE DEPENDENCE OF MUSCLE TENSION ON STIMULUS INTERPULSE INTERVAL AND MUSCLE LENGTH. Patrick L. Crapo. Case Western Reserve University, Cleveland, Ohio 44106.

Quantitative assessment of the contribution of temporal summation to total muscle force would be improved by the availability of a real-time model depicting the firing pattern for isotonic and isometric contractions. Models that are linear in the frequency domain (Milner-Brown, Stein and Yemm, J. Physiol. 230, 371, 1973) are only applicable over restrictive ranges of stimulus intensities. In the present experiments, the relationship between force and firing rate was determined for a wide range of lengths (-20 to -4 mm with respect to maximum). The model is currently being used in studies of force modulation under physiological conditions and in the design of control schemes for orthopaedic stimulation of muscles. (Funded by NIH, NINCDS Contract Number 1NO1-NS-2-2314).

DIFFERENCES IN THE REFLEX EFFECTS OF DIGITAL NERVE STIMULATION ON THE FIRING OF LOW AND HIGH THRESHOLD MOTOR UNITS IN HUMAN FIRST DORSAL INTEROSSEUS MUSCLE. A.K. Datta* and J.A. Stephenson* (SPM: A. Taylor) Sherwood Institute of Physiology, St. Thomas's Hospital Medical School, London, ENGLAND.

Physiological observations in both that the pattern of recruitment of motor units can be altered by cutaneous stimulation (1,2). During controlled ramp contractions of low dorsal intersosseus muscle (D1), cutaneous stimulation of the digital nerves of the index finger raises the recruitment threshold of units normally recruited at contraction strengths >1.5N and lowers the recruitment threshold of units normally recruited at strengths >1.5N. One explanation for this finding would be that cutaneous stimulation has an overall excitatory effect on high threshold units and an inhibitory effect on low threshold units. To investigate this hypothesis we have performed the following experiment.

Subjects were required to maintain a contraction of D1 such that the motor unit under study fired steadily at 10pps. 80ms after every third unit action potential the digital nerves of the index finger were stimulated with double shocks at 300μs interval for perception. Histograms were then constructed of the intervals between the two motor unit spikes following each stimulus. Given an afferent plus efferent conduction delay of 30ms the reflex effects of the stimulus begin some 10ms after the start of each measured interval. We can assume that if the overall reflex effect of the stimulus begins some 10ms after the start of each measured interval to be prolonged.

All units recruited at contraction strengths >1.5N had their measured inter spike interval shortened by digital nerve stimulation which was performed immediately before each stimulus presentation (100μs). The excitatory drive to these motor units had been increased by the stimulus. The net reflex effect of cutaneous stimulation for the units not recruited at contraction strengths >1.5N had little effect, as the duration of the reflex effect of the stimulus for units Recruited at contraction strengths <1.5N and modifies the properties of the reflex effects (1). The results show that cutaneous stimulation has an overall excitatory effect on high threshold units and an inhibitory effect on low threshold units. To investigate this hypothesis we have performed the following experiment.

We conclude that the changes in the pattern of recruitment produced by cutaneous stimulation in man as in cat can be attributed to differences in the reflex connections of motor units which are related to motor unit mechanical properties.


ULTRASTRUCTURAL CHARACTERIZATION OF CEREBELLAR TERMINALS ON RUBROSPINAL NEURONS IN RABBIT. A COMBINED ELECTROMICROSCOPIC STUDY USING ANTIGERODERE AND RETROGRADE AXOPLASMIC TRANSPORT. Jan J. Dekker, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Lesions of the precentral cortex in humans and infrahuman primates result in a condition termed hemiparesis, initially hypotonic and paretic, the animals were given 100 μCi/kg of 2-DG intravenously. Forty-five minutes later the animals were sacrificed; the brains were removed and sectioned for autoradiography of ventral root filaments. Some data published previously by other investigators was also used.

The following analytical model describes the results:

\[ F = F_0 + \frac{a}{b + IP} \]

where F is muscle force and L is length. F_0, a and b are empirically derived constants.

This model is currently being used in studies of force modulation under physiological conditions and in the design of control schemes for orthopaedic stimulation of muscles. (Funded by NIH, NINCDS Contract Number 1NO1-NS-2-2314).
EFFECTS OF SPINAL CORD STIMULATION ON SEGMENTAL REFLEXES IN MAN
Milan R. Dimitrijevic, L. Donald Lehnhohl* and Arthur M. Sherwood*

The Institute for Rehabilitation and Research, Houston, Tx. 77030

Neurophysiological follow-up studies are in progress in 15 patients with implanted systems for epidural spinal cord stimulation. These patients were treated because of severe axial problems. Somatosensory evoked potentials, tendon jerks, vibratory reflexes and polyvolelectromyographic recordings of spinal reflexes and their dependence on bradycardia were obtained in all patients. Small amplitude, damped oscillations were found at approximately 3, 6 and 12 month intervals post-implant. Evaluation of the evoked spinal cord and somatosensory potentials, and the interaction of tendon jerks and vibration revealed no significant changes in the neurophysiological characteristics of the four patients evaluated by this time. Overall, there was a noticeable decrease in the electromyographic features of spasticity and evidence of improved suprasegmental control in these patients.

REFLEX EMG ACTIVITY IN PARKINSONIAN PATIENTS. Joel R. Dufresne and John P. Soechting. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, MN 55455

The myotatic reflexes of Parkinsonian patients were tested by applying pseudo-random torques about their elbow joints. Rectified, surface EMG activity was obtained from the biceps and triceps muscles. A description of the reflex motor output was provided by impulse response functions giving the average EMG response to a 20 ms pulse of torque. Two populations of Parkinsonian subjects could be distinguished on the basis of these impulse response functions. The first group yielded responses similar to those for normal adult subjects. Subjects in this group were observed to have little rigidity. In general, their data could be fit by a simple linear model relating EMG activity to forearm position, velocity, and acceleration.

The EMG impulse response functions for patients in the second group were significantly different, in that they contained a large damped oscillation at a frequency of 10-12 Hz. This oscillation was not present in normal subjects or in the first group of Parkinsonian patients. Moreover, their frequency was much higher than that for typical Parkinsonian tremor (3-5 Hz). They were also observed to outlast any large transients in the impulse response functions for forearm velocity and acceleration. Patients in this group had a significantly high level of rigidity in their "off" state. However, the oscillations were actually greater during the "on" state, when rigidity had been effectively suppressed by L-Dopa.

These results confirm and extend previous work showing that patients with rigidity had exaggerated M2 responses. They suggest that: (1) While oscillations in the EMG impulse response functions could be correlated with the clinical observation of "off" state rigidity, rigidity itself was not directly responsible for them. (2) The oscillations appear to be triggered, rather than sustained, by kinematic feedback from peripheral receptors.

(Facility work was supported by NIH grant NS-15018 and by a grant from the American Parkinson Disease Association.)


Electromyograms (EMG)'s show that stingrays locomote with a rostral to caudal wave of elevation and depression of the pectoral fins at each second level. At the actual level the anterior and central musculature alternates. Increased swimming velocity, due to exteroceptive stimulation, produces decreased cycle times and increased EMG activity. Although the overall cycle changes, the duration of bursts relationship remains the same. Animals restrained by chronically implanted vertebral clamps swim with the same cycle times and EMG patterns as freely swimming animals. Unilateral or bilateral injections of morphine, in the moderate range, perturb the mesencephalic/diencephalic junction locomote spontaneously while animals with high spinal transections do not. The EMG pattern recorded from deafferentated stingrays is the same as that recorded from intact animals.

In this report, compound neuronal activity was investigated in the restrained, deafferentated preparation. A cuff electrode was attached to the peripheral nerve innervating the dorsal fin muscles. Simultaneous recordings were made from the cuff and from bipolar EMG electrodes in muscle of the same segmental level. Rhythmic bursts of neural activity were recorded corresponding to the EMG bursts during spontaneous locomotion. The cycle times measured from either neuromuscular or EMG's were approximately 0.9 s. This is within the range observed in different types of preparations. The animals were then immobilized with curare and artificially respired. The dose was sufficient to block both spontaneous EMG activity and EMG evoked by stimulation of the nerve in the cuff electrode. Following immobilization, spontaneous rhythmic discharges continued in the neurelem. If these discharges stopped, they could be elicited by exteroceptive stimulation or electrical stimulation of the rostral midbrain tegmentum. In all cases the rhythmic activity was also evident in the EMG impulses. Such stimulation also evokes normal locomotor movements in nonparalyzed, decerebrated animals. Rhythmic EMG or neurogram bursts often continued for several cycles after cessation of the stimulus.

The observation that normal locomotor rhythms can be recorded from the motor nerve after immobilization establishes fictive locomotion in this preparation. This particular preparation, immobilized, stimulated stingray thus provides a good experimental model for further investigation of vertebrate locomotion.

(Supported by a grant from the Muscular Dystrophy Assn. and from the National Institutes of Health NS 11525.)

REFLEX EMG ACTIVITY IN PARKINSONIAN PATIENTS. Joel R. Dufresne and John P. Soechting. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, MN 55455

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As part of an investigation of the physiology and behavior of the flexion reflex in spinal cat the present study was designed to test motor unit recruitment order in the tibialis anterior (TA) muscle. The experimental preparation was the unanesthetized, decerebrated cat with a T-10 spinal transection made either two weeks before or immediately after spinal transection. Motor unit EMG was recorded by means of fine needle electrode inserted into the belly of the TA muscle. Electrical stimulation of the cutaneous superficial peroneal or saphenous nerves was used to evoke flexion TA muscle activation. Cutaneous nerve stimulus intensities were below those required to activate cutaneous C fibers.

The reflex discharges of pairs of motor units were monitored simultaneously and recruitment order was determined either as a function of varying stimulus intensity or by determining the probability of firing at a constant stimulus intensity. In the flexion reflex of acute spinal cats recruitment order was generally in order of increasing EMG spike amplitude. Such a relationship was observed in 75% of motor unit pairs with reversal in about 20% of the cases. These results are significantly different from chance. In contrast, recruitment in chronic spinal cats was very different with large EMG spikes often being recruited with a greater probability and at stimulus intensities lower than units with small EMG spikes (in over 55% of motor unit pairs). Recruitment orders for pairs of units determined using superficial personal nerve stimulation were, with few exceptions, the same as those determined using cutaneous nerve stimulation for both acute and chronic spinal cats.

Studies from other laboratories indicate that a direct relationship exists between motoneuron size and EMG spike amplitude. If this relationship applies to the present experiments, the results suggest that the majority of TA alpha motoneurons are recruited in an orderly manner. However, both small and large EMG spikes are activated in fission reflexes and not just the large EMGs seen in chronic spinal cat. Supported by NSF Grant BRS 77-23845.

The role played by motor unit activity in determining the biochemical and physiological properties of the spinal cord is thought to be critical. Lower spinal tracts have resulted in a transformation of both the histochemical profile of some fibers in the soleus (SOL) muscle of the cat. Arch. Neurol. 15: 652, 1968) and the contractile properties of the whole SOL in cats (Buller, et al, J. Physiol. 150:399, 1966). It has also been hypothesized that the observation of the time course of SOL, after cord transection, is due to a virtual elimination of motoneuron discharge (Gallego, et al, J. Physiol. 281:253, 1978).

To study the activity of neurons 2 weeks old, eight 2-week old kittens and six 12-week old cats were completely spinalized at the level of C2. Each cat was later subjected to an operant conditioning protocol in which the neurons were activated with back-pedaling movements. The onset, duration and intensity of activity were noted, but not of the same order as has been reported for peak EMG amplitudes (M. Smith et al, J. Neurophys. 40:503-513, 1977). It is concluded that the individual muscular components may make differential contributions to the overall force at the tendocalcaneus which reflect a division of labor in the complex. Supported by Grant AM19916-02.


Unlike the findings in the mammalian spinal cord, there has been neither anatomical nor physiological evidence supporting the presence of axon collaterals from cranial motoneurons. In particular, extracellular axonotomies and intracellular injections of extravascular motoneurons have failed to detect recurrent synaptic effects. Nevertheless, intracellular injections of horseradish peroxidase into the medial and four extracranial motoneurons has demonstrated the existence of axon collaterals in every case. In seven cats, both the dorsal and ventral, as well as rostral and caudal subnuclei of the oculomotor nucleus have been histochemically identified fiber types from a slow-to-fast twitch profile occurred in the SOL, but the percentage was not consistent and did not have the degree of homogeneity seen in the physiological properties. Fatigue properties of the SOL and MG motor units remained within normal ranges for all groups tested.

This data suggests that absence of activity cannot explain the shorter CT for the SOL after transection, since this occurs even when the motor units are involved in considerable amounts of activity during locomotion.

Supported by a grant from the Easter Seal Foundation.


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Supported by a grant from the Easter Seal Foundation.

SHORT TIME SCALE CORRELATIONS BETWEEN DISCHARGES OF MEDULLARY RESPIRATORY NEURONS. J.L. Feldman, D. Sonmer* and M.I. Cohen. Dept. of Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 14461 and Dept. of Physiology and Anesthesia, Northwestern Univ., Chicago, Ill. 60611.

The intersections on a short time scale among inspiratory (I) neurons and among expiratory (E) neurons of the cat medulla have been studied by analyzing simultaneous recordings of spike activity from two or more neurons. Microelectrodes were used to record activity in three regions: a) the ventrolateral nucleus of the solitary tract (vNTS), containing I neurons almost exclusively; b) the rostral portion of the ventral respiratory group (vRG), in the ventrolateral medulla rostral to the obex containing mostly I neurons; and c) the caudal portion of the vRG (cRG), containing mainly E neurons. Crosscorrelation histograms (CCHs) between activities of two I neurons or two E neurons were used to ascertain the existence of correlated discharges on a short time scale (ca. 1 msec), as indicated by a sharp peak near zero lag in the CCH. Adjacent neuron pairs (recorded with the same electrode) have a high incidence of such correlated discharges: they were present in 16/26 pairs of I neurons and 28/45 pairs of E neurons. In contrast, such correlations were extremely rare for pairs of distant neurons (recorded with two microelectrodes on opposite sides of the medulla): only 1/43 I neuron distant pairs had a clear cut peak in the CCH and none of the 97 (9/97) E neuron distant pairs had such a peak. However, 19/43 I neuron adjacent pairs had common high-frequency oscillations (HFOs), with period 9-17 msec. These results indicate that: a) within local clusters of I or E neurons there is a high incidence of correlations for recorded inputs; b) only a small fraction of distant neuron pairs are disynaptically connected. Since respiratory neurons of a particular discharge type are ultimately connected, as indicated by synchronization of firing on a time scale of seconds or tens of milliseconds (HFOs) the coordination of activity between different regions may reflect the existence of functional synaptic connections between specialized subpopulations. (Supported by N.I.H. Grant NS-20860).
Mean rising slopes of the earlier fast hyperpolarization indicates that postsynaptic inhibition polarizes. Clear evidence of IPSPs during the but also IPSPs are involved in the fast hyperpolarization; application of hyperpolarizing current results in an increase in amplitude. Hyperpolarization was not affected, but the later slow hyperpolarization coincided with that of the abrupt disappearance of the nerve activity. After electrophoretic injection of C1 ions into the cell, the earlier fast hyperpolarization was not affected, but the later slow hyperpolarization was reversed by depolarization. Mean rising slopes of the earlier fast hyperpolarizations before and after C1 ion injection were not significantly different in the motoneurons whose resting membrane potentials and frequencies during nystagmus were stable. Application of depolarizing current through the recording electrode filled with 2M K-citrate revealed a reduction of amplitude in the fast hyperpolarization; application of hyperpolarizing current resulted in an increase in its amplitude.

These results indicate that disfacilitation is primarily responsible for the quick cessation of the axial rectus motoneurons activity; contributions of IPSPs to the fast hyperpolarization must be very small, if present at all. This pattern of activity differs from the synaptic mechanism in the anterior rectus motoneurons in which not only disfacilitation but also IPSPs are involved in the fast hyperpolarization. Clear evidence during the later slow hyperpolarization of medial rectus motoneurons indicates that postsynaptic inhibition does contribute to this phase of activity, however.
impaired. Therefore, measurements of speed were used to indicate degree of impairment and the variable distance between them, 2" wide runways and 12" wide area 5.

Injections into the caudal two-thirds of the RN labeled cerebellar rubral neurons are currently under investigation. (Supported by NIH Grant NS-10183).

1242 HIERARCHICAL CONTROL OF LOCOMOTOR RECOVERY FROM THE EFFECTS OF DORSAL ROOT LESIONS. Michael E. Goldberg, Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Recovery of motor function may be due not to the mass action of undamaged pathways but to a controlled process in which the contribution of each pathway is somehow ranked or weighted; i.e. the recovery is regulated hierarchically. If this explanation were correct, one would be able to determine that the contribution of each pathway is more important than another recovery in a particular lesion. The role of descending systems in recovery was assessed using the horizontal and forelimb movement unit number method. (1) LEMS: In this preparation in which: a) all 1-5 roots were cut, or 2) the L6 root was spared (spared root preparation) and were also compared with the effects of hemisection alone. Rat hemisection labeled locomotor tasks involving timed traverse of 9 ft. long runways. These included an obstacle course, boards with randomly placed holes of varying sizes, paths, and more. The outcome of experiments with distances between them, 2" wide runways and 12" wide boards which were used as controls for locomotor speed. The more difficult tasks limited the time of a traverse in normal and lesioned animals by increasing the number of 'mistakes' the animals made (i.e. by requiring greater accuracy). Therefore, measurements of speed were used to indicate degree of impaired and course of recovery from it. L5-L7 rhytontes sparing L6 produced a mild deficit on all runways except the 12" wide board on which no deficit was seen. Recovery began when tactile placing returning at 7 days and was "complete" by 6 weeks. In another group of cats, L1 hemisection produced a severe deficit which began to show recovery on the 12" board on day 19 whereas on the other days at day 9. Recovery was "complete" by day 6 weeks. If ipsilateral hemisection was added in the recovered spared-root animals, a severe deficit resulted which also showed recovery on the 12" board beginning at 2d and on the other runways beginning on day 8 and subsequent placing recovery. Recovery was "complete" by 6 weeks. In contrast, hemisection produced in the ipsilateral differentiated factor replenishes all recovery permanently. The recovery in deafferented cats is therefore dependent on the ipsilateral descending systems, but recovery in spared root animals depends on contralateral systems. Since sparing a root (Goldberger and Murray 1978) also: (1) blocks sprouting of descending systems and 2) prevents the development of hyperactivity of descending (e.g. corticospinal) reflex effects both of which are seen in deafferented cats, the results suggest: a) an inequality in importance of the spared root and descending systems in mediation of recovery and that b) recovery and sprouting show similar response to a particular combination of lesions. Supported by: NIH Grant NS-13768.

1243 ABSENCE OF CROSS-ARTS PLASTICITY IN THE VESTIBULO-OCULAR REFLEX. Lionel O. Greene, Jr. and Laurence R. Young. MIT, Department of Aero/Astro, Center for Space Research, Cambridge, MA 02139.

The vestibulo-ocular reflex (VOR) plasticity shown following the wearing of optically reversing prisms represents a change in gain in afferrors originating in semicircular canals lying in the same anatomical planes, but not a transfer of innervation to the extraocular muscles from canals situated in differing orientations. The vestibular response to visual inputs requiring compensatory ocular movement across the semicircular canal planes was monitored in present experiments by fitting two rhesus monkeys with dome prism spectacles that caused vertical visual field movements with vertical head movements. Eye movements (velocity) in the horizontal and sagittal plane was assessed during passive or voluntary movements (accelerations to 30,45,60, and 90°/s, 25 s continuous angular eye movements). Very few very few ABN in the proximal forelimb region of area 4 and in both banks of the presylvian sulcus, which are thought to correspond, respectively, to the supplementary motor area and the frontal eye field in the monkey. This implies that monkey features. If this explanation were correct, one would be able to determine that the units previously recorded in the monkey abducens nucleus, and not antidromically identified, are not all motor neurons and the units is biased toward shorter phase-lead neurons.

To answer this question, we made phase measurements of neural discharge in animals implanted with bipolar electrodes near the abducens nucleus. Two methods of measurement were used: (1) Discharges of antidromically identified ABN were recorded while the animals were being exposed to rotational movements with frequencies of 0.1-1 Hz. At 0.25 Hz, motoneurons lead the eye by 24°, and the phase lead was not affected when the nerve fibers were driven antidromically from the 6th nerve. The monkeys exhibited an average lead of about 33°. Both types of neurones increase discharge during upward gaze, and during upward gaze.

The 24° average phase lead for the alert cat ABN approximates the reported 18° monkey phase lead. The 2° lead reported for deafferented cat ABN is most likely due to the preparation and its state of alertness. Supported by the National Institutes of Health grant EY-00955 and Training Grant T32 EY07379-02.

An inherited canine neuromuscular disorder is characterized by general muscular weakness, muscular atrophy, and an intolerance to cold and exercise stress. The disorder is inherited as an autosomal recessive disease. Clinically, the question of whether the muscular weakness is explained by the muscular atrophy or an occlusion of the peripheral target circuits. In fact, it is likely that the massive dendritic tree of a functional segregation based on the peripheral target structure of collateral projections.

(Supported by NIH grants RS05151, 55465, GM07125, and the Muscular Dystrophy Associations of America, Inc.)


In search for a general principle governing the anatomical organization of the deeper or motor layers of the superior colliculus, we analyzed the distribution of reticular cells which give rise to several functionally distinct projections and compared their laminar and spatial arrangement. The histochemical method of de Olmos ('77) for labeling collicular cells in a similar distribution, were found predominantly in the caudal and lateral portions of the colliculus. In several additional animals HRP deposits were made in cervically-projecting areas of the reticular formation below the nucleus. In addition, dendrites of both populations curved around the facial genu, especially laterally to the MLF. We suggest that the morphological differences in a causal collateral that courses in the contralateral MLF towards the prepositus. The dendritic trees of all Abd Mns exhibited more extensively than those of Int Ns, especially at secondary and tertiary levels. Dendrites of Abd Mns often spanned the entire dorsal-ventral extent of its medial border. Every Int axon headed toward the contralateral MLF before curving rostrally towards the IIIrd nucleus; but 30% exhibited a curving rostrally towards the IIIrd nucleus before developing the retrogradely transported HRP enzyme was used, and the position of labeled cells was recorded with the aid of an x-y plotter. In five cats iontophoretic HRP deposits were made in cervically-projecting areas of the reticular formation and compared their laminar and spatial arrangement. All neurons received dendromatric collaterals within the Abd nucleus demonstrates that horizontal dendritic tree of Abd Mns were always extended more dorsomedially toward the MLF. We conclude that the absence of any recurrent axon collateral formation below the nucleus. In addition, dendrites of both populations curved around the facial genu, especially laterally toward, but not entering, the medial vestibular nucleus. The dendrites of Abd Mns were always extended more dorsomedially toward the nucleus. We suggest that the morphological differences in soma-dendritic profiles (i.e., size) are responsible, in part, for the quantitatively different physiological responses in Abd Mns and Int Ns. We conclude that any recurrent axon collaterals within the Abd nucleus demonstrates that horizontal conjugate gaze signals are determined individually by all neurons within the nucleus and no single circuit is the cause of which is shared by the comparatively smaller dendritic trees of Int Ns and medial rectus Ns. Supported by Grants EY-02007 and EY-01670.
A comparison of the discharge patterns of motor cortex and spinal mental motorneurons. During the voluntary tracking of low frequency perturbations, the task is performed principally by small units, which have larger twitch tensions; their firing rates are close followed the whole muscle EMG during walking. At higher perturbation frequencies (0.6-2.0 Hz), these small units discharged at near constant rates, which appear to be near maximal charge at near constant rates. The motor neuron was recruited as the rectified/smoothed muscle EMG crossed a reproducible level, and its firing rate closely followed the whole muscle EMG during walking. If so, then PAT might be considered the physiological substrate for the tremors produced by fatigue.

ALTERATION OF PHYSIOLOGICAL ACTION TREMOR BY FATIGUE. Paul A. Iaizzo*, Robert E. Pozza, Roger W. Petry* (SPON: D.J. Forbes) Department of Physiology, University of Minnesota, Duluth, School of Medicine.

An action tremor which accompanies voluntary movement in normal subjects has been called Physiologic Action Tremor (PAT). Since it has been observed in patients with tremor of the limbs can be observed, in extremely fatigued subjects, the present study was undertaken to see if fatigue altered the frequency and/or amplitude of PAT. Fatigued subjects and controls were monitored during walking, and to characterize motor units by frequency and amplitude changes using a PDP-12 digital computer. The fatigue state was produced by asking the subject to run a distance which the subject considered moderately fatiguing. Analysis of the data showed that the frequency range of PAT in the non-fatigued subject versus the tremor seen when a subject was fatigued, fall in the same frequency range of 3-8 Hz. There was a significant difference (p<.05) in the amplitude of the acceleration record as well as the EMG signals in the fatigued subject versus the tremor seen when a subject was fatigued. Fatigued subjects have a higher frequency and/or amplitude of PAT. If so, then PAT might be considered the physiological substrate for the tremors produced by fatigue.


Some patients with impaired supranuclear oculomotor control have pathologic fixation eye movements. A large number of these cases can be explained by assuming that the oculomotor system is trying to maintain an adequate gaze on a target. Small amplitude saccades and slow drifts are widely known to occur in normal subjects in similar test situations. The EOG from control subjects were recorded using a purkinje image eyetracker and in addition to saccades and slow drift, small amplitude square waves, polyphasic square waves, fast saccadic movements, and vertical nystagmus were recorded. Square waves consisted of an initial disruptive saccade which, after an intersaccade interval less than 350 msec., was followed by a second corrective saccade returning the eye toward the intended position. The mean amplitude of square waves (19.7', SD 10.4) and polyphasic square waves (40.0', SD 16.7) was significantly greater (p<.001) than horizontal fixation saccades (9.2', SD 3.3). Intersaccade intervals of square wave varied from 50 msec. to 350 msec. Square waves with an interval less than 200 msec. were greater in amplitude (p<.004) than matched paired squares with 250-350 msec. intervals, suggesting that the corrective saccade occurred sooner when the initial saccade produced a large gaze deviation. The mean amplitude of the first saccade of polyphasic square waves (25.8', SD 9.9) was similar to the mean square wave amplitude, however the mean amplitude of the second saccade (48.6', SD 18.4) was significantly greater (p<.016). The mean duration of the first intersaccade interval of polyphasias was also shorter than square waves (p<.016), while the last polyphasic interval was the same. Polyphasias may be produced by a coupling mechanism similar to square waves, however the occurrence of a second square wave after a first square wave shows the position of intended gaze, requiring additional corrective saccades.

Two zones in the pontine tegmentum have been identified with distinct oculomotor functions in cats and monkeys on the basis of recordings from single units during eye movements: 1) a paramedian zone containing "burst" and "burst-tonic" units that increase their firing rates shortly before eye movements, and 2) a more medial zone near the level of the VIth nerve rootlets containing "omnipause" units that cease firing before a saccade occurs. We have attempted to distinguish between the efferent connections of these two pre-oculomotor regions by means of the autoradiographic technique. In 3 cats the "pauser region" was identified electrically into the mesencephalic tegmentum. Regions of the central gray substance, particularly near the IIIrd and IVth nerve nuclei, were variably labelled, somewhat more so in cases of midline than paramedian injection. Labelling of the abducens-periabducens region also appeared but could not be interpreted adequately because the deposits were too close by.

Two main generalizations arise from this anatomical data. (1) Every segment of the lumbosacral cord has motor pools innervating stance muscles and motor pools innervating swing muscles; thus, the entire step cycle is represented in each segment. (2) There is a longitudinal continuity of motor nuclei that innervate muscles with a similar activity pattern.

Supported in part by PHS Grant RNS 14066 to N. Hollyday. RJ is a Medical Scientist Trainee supported by PHS Grant #132-GMO-7281.

Withdrawn by Author

Eff erent Projections of the Pontine Oculomotor Pauser Region in the Chick. C.R.S. Kambo, T. F. Laquinta, and A.M. Craighead, Dept. of Psychol., MIT, Cambridge, MA 02139 and Dept. of Physiol. and Bio­

physic, Univ. of Washington, Seattle, WA 98105.

The functional implications of these differences remain to be investigated. While the anatomical findings cannot definitively establish the projections of functionally defined cell groups, these differences suggest that medial "pauser region" and paramedian teg­

mental nuclei indeed have distinct efferent connections. In particular, the findings suggest that the medial pauser region of the pons may have direct efferent connections with both vertical and hori­

zontal saccade-generating mechanisms and with cerebellar-

precerbellar circuitry as well.

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Organization of Reflex Responses to Ankle Displacement in Human Leg and Arm Muscles. R.E. Kearney and C.W.Y. Chan, Biomedical Engineering Unit and Aviation Medical Research Unit, McGill University, Montreal, Quebec, Canada.

In a previous study (1) we demonstrated that cutaneous stimu­

lation of the human foot evokes systematic reflex changes in muscles of both the ankle and arm. The present study extends this work to consider the nature of the responses elicited by ankle displacement.

First, the responses of the ankle muscles are distinctly asym­

metric. Dorsiflexion of the ankle evoked a strong, short latency (40 ms) excitation of G, attributable to the monosynaptic la path­

way. This was followed by a period of reduced activity, and then a return to normal. The TA displayed an exactly reciprocal pattern of response, in which an early decrease in activity was followed by a period of excitation. In contrast, plantarflexion of the ankle evoked a weaker, short latency excitation of TA followed by a second, often larger, period of excitation lasting from about 80 - 100 ms. Furthermore, the G response did not display any significant reciprocal inhibition. It is possible that the asymmetry of the response reflects differences in the relative importance of cutaneous and muscle afferents in the two muscles. The functional implications of these differences remain to be determined.

The second major finding was that ankle displacement evoked large and systematic reflex responses in TA but not BB. Thus dorsiflexing displacements in the ankle result in a decreased TA activity at a latency of about 70 ms while there was no significant change in BB activity. In contrast, plantarflexion of the ankle evoked a weak, short latency excitation of TA followed by a decrease in activity. Occasionally, small increases in BB activity were also noted. The large size and short latency of the BB responses indicate that they may have important functional roles to play - perhaps in the control of locomotion.


Supported by a grant from the Medical Research Council of Canada.

WITHDRAWN

MOTOR SYSTEMS

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PONTINE CONTROL OF SACCADIC TRAJECTORIES: MICROSTIMULATION AND SINGLE UNIT STUDIES. W.H. King, A.P. Puchalski, W. Becker, and C-Johannson

University of Washington, Seattle, WA 98195 & Université Laval, Québec, Canada

Although it has long been thought that saccadic eye movements are preprogrammed, Robinson (1) has recently suggested that the saccadic trajectory is under continous control by pontine circuits. To test both this hypothesis and to determine the neural elements involved in such circuitry, we attempted to interrupt saccades transmitted mid-flight by delivering stimulus trains to various brain stem loci between the abducens and oculomotor nuclei. Cathodal stimulus trains (0.1 msec bipolar pulses at 300 Hz with 10-35 msec train durations) were triggered to occur as early as 3 msec after the onset of randomly selected saccades elicited in the trained monkey. At certain sites near the midline, currents as low as 20-50 µA caused saccades to decelerate within 11 msec of the train onset. Within 11 msec after the train ended, the saccade either resumed its course (perturbed saccade) or was terminated (truncated saccade). A perturbed saccade appeared to be a single eye movement, and in most cases was as accurate as a control saccade to the same target. In contrast, the truncated saccade always fell short of the target and was followed by a second saccade, often after latencies of less than 50 msec. For long stimulus trains, the second saccade overcompensated the truncation phase of control saccades to the same target. If the stimulus train ended after the estimated acceleratory phase of the eye movement, this second saccade was truncated.

Preliminary reconstructions of successful penetrations indicate that sites where weak stimulation caused saccade interruptions were invariably confined to within ±1 mm of the midline throughout the core of brain stem which extended from the rostral pole of the lateral from about 5 to 7 mm ventral to the trochlear nuclei. Simple unit recordings obtained through the stimulating microelectrode indicated that sites where omnipause neurons (which ceased firing for saccades in all directions) were encountered always produced saccade interruption at lower currents, but interruption was obtained also at sites without omnipause neurons. However, at virtually every effective site some form of saccade related activity was found.

Generally, these results seem consistent with Robinson's "bang-bang" model although modifications will be necessary to account for certain characteristics of interrupted saccades.


PROJECTIONS FROM MESENCEPHALON TO THE INFERIOR OLIVARY COMPLEX

CATS AND MONKEYS. Pierre Langeller*, Raymond Marchand*, René

Boucher and Louis J. Poitier, Laboratoires de neurobiologie, Pav. Notre-Dame, 2057 ave de la Vitré, Québec, Qué., G1J 5B1.

In three other cats and one monkey, 3H leucine was injected into the medial portion of the superior colliculus. In agreement with Weber J.T. et al (Brain Research, 144 (1978) 369-377) we found the labeled amino acid into the dorsal part of contra­

lateral rubro-olivary pathway. Distribution of silver grains in the ipsilateral dorsal lamella with injections in the vicinity of the periaqueductal gray. (Supported by M.R.C. of Canada)


Recent studies of motor coordination have emphasized the importance of the mechanical properties of muscle in the control of movement. In the present study, we investigated short term changes in muscle properties by measuring the response of the intact human elbow joint to mechanical perturbations. The resistance of the intact joint to such displacements reflects contributions from the muscles and passive tissues spanning the elbow. In order to study the effects of both muscular activation and movement on joint properties we studied three conditions: (1) voluntary isometric contraction,(2) passive movement, and (3) voluntary move­

ment.

In the isometric condition, the subject maintained a con­

stant arm position against various loads. In the passive movement condition the subject's arm was moved at a constant velocity. Finally, the voluntary movement condition combined muscular activation with movement. During all three conditions a small amplitude, high frequency (3-30 Hz) sinusoidal perturbation was applied about the elbow joint. Joint impedance was derived from the resistance of the elbow to this superimposed displacement. We obtained continuous measures of joint impedance, joint angle, and the biceps and triceps EMG's.

During isometric contraction, joint impedance increased monotonically with increasing muscular activation (estimated by EMG). In contrast, during passive arm movements joint impedance decreased markedly. This was true even for very slow passive movements when no change in muscular activation could be detected. Finally, voluntary movements resulted in changes in joint impedance which were consistent with the combined effects of muscular activation and movement.

We attribute changes in joint impedance to variations in the properties of the muscles acting about the joint. We conclude that length changes (whether active or passive) and neural activation have opposing effects on the mechanical properties of muscle. These results will be discussed in relation to the well-characterized effects of length change and activation on the mechanical properties of isolated muscle.

Supported by NIHGS grant T32-AM07404, NIH grant NS09934, and NASA grant NGR 22-009-798.
The reflex EMG response to perturbation of an extremity consists of an early component (M1) and late components (M2, M3). The origins of the late components have been the subject of much recent controversy. Several lines of evidence, including the long latencies of the late components, support the hypothesis that the late components are at least partially mediated by a transcortical feedback loop. However, assigning specific functions to these late components requires additional experiments. The present study was designed to examine the latencies of the EMG responses to generate randomly timed displacements of the wrist joint. EMG responses from the wrist flexors were rectified and averaged and the integrated EMG activity was measured over the M1 interval (30-55 msec, following onset of the imposed load) and over the M2-M3 interval, (55-90 msec). When the displacement was stopped prior to 45 msec, 43% of the late EMG components beyond 55 msec were absent. As the duration of the displacement was increased beyond a critical interval (between 50-55 msec), there was a reduction in the number of the late responses. Further, the duration of the late components was monotonically related to the duration of the imposed displacement beyond the critical period for an M1 response (30-55 msec) in darkness was greater than 1.5. The rest of the sample consisted of an early component (M1) and one or more late components. In addition, there were no differences in phase shift and latencies to various skin areas of the limb during walking. These firing patterns have been obtained through the use of an expanded version of the technique for the separation of superimposed action potential trains reported at the 8th Annual Meeting of the Society for Neuroscience. Three channels of EMG signal, one bipolar and two monopolar, are recorded from a modified DISA bipolar needle electrode. Adductors were chosen for the EMG signal since it is easier to reduce the duration of the action potentials, and consequently the degree of superposition. The EMG signal is decomposed into its constituent action potential trains using a computer algorithm. Using this technique, the firing patterns of typically three to six motor units can be obtained throughout an entire contraction with usually less than 20% error. Statistical analysis performed on these firing patterns indicates that concurrently-active motor units tend to rapidly modulate their firing rates to a greater extent than motor units activated or modulated during a constant force level is increased. Also, in the FDI of one subject, three motor units were observed to lock into exact synchronization for a period greater than 1.5 seconds during an isometric contraction at 60% of its maximal projection. These motor units were not firing synchronously, however, both their firing rates and modulation of these rates were similar. The latencies to these firing patterns exhibit the greatest amount of firing rate modulation at high force levels. This discharge behavior of motor units suggests that the number of motor units recruited and their overall firing rates supply the bias of a contraction level, and that small fluctuations about this force are achieved through the simultaneous changes in the firing rates of all active motor units. This scheme has great appeal from a systems control viewpoint. (Supported in part by NIH grant EY 16665, and by a joint grant from the British Heart Foundation and the C.A. Dana and the Foundation.)
ACTIVITY OF MULTIPLE INDIVIDUAL MOTOR UNITS IN CATS DURING LOCOMOTION. W.B. Marks. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Arrays of wires are implanted in the medial gastrocnemius muscle of the cat's hindlimb. Recording tips are spaced closely enough apart that, for different units, the ratios of amplitudes in the leads differ. The number of active units is held within the limits of the range of the multichannel filter (Roberts & Hartline, Brain Res. 94:141, 1977). We will attempt to derive from such multiunit responses, the "muscle activity level," which is the fraction of units whose amplitudes rise while that of others is falling, or if in general all the units are active and have the same firing rate. Changes in their waveform in a few channels caused by movement. In addition, the lead array is stabilized by attaching it to the surface tendon; the leads terminate just below the surface, the origin of the fibers. When two or more unit waveforms overlap in time and superimpose, they are resolved using a digital multichannel filter (Roberts & Hartline, Brain Res. 94:141, 1975) which forms linear combinations at multiple delays from all the channels, each combination orthogonal to all but a chosen multichannel unit waveform. Multi-lead recording appears to be functional at high levels of muscle activation, since the extra channels enable the filter to resolve more overlapping impulses.

Thus far we have followed the activity of up to five motor units of the medial gastrocnemius muscle during walking, using 8 leads spaced around 300 microns apart and implanted in the dorsal muscles of the hindlimb. The lead array is stabilized by attaching it to the surface tendon; the leads terminate just below the surface, the origin of the fibers. When two or more unit waveforms overlap in time and superimpose, they are resolved using a digital multichannel filter (Roberts & Hartline, Brain Res. 94:141, 1975) which forms linear combinations at multiple delays from all the channels, each combination orthogonal to all but a chosen multichannel unit waveform. Multi-lead recording appears to be functional at high levels of muscle activation, since the extra channels enable the filter to resolve more overlapping impulses.

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was examined in each of the 20 conditioning and 20 sensitization trials. Each unit must have fired at least 30 sec after each presentation of the CS alone. In both paradigms the interval between CS presentations was 60 sec. Five pre-acquisition CS-alone trials were presented before the first CS train. During conditioning or sensitization was based upon the discharge behavior during 15 acquisition trials for both conditioning and sensitization. The criteria were such that each unit must have fired at least three to five weeks after plating.

Our results have demonstrated that myotubes cultured from normal biopsies have abnormalities in some of their fundaments, such as the appearance of striations, and were studied with the EM or light microscope level which makes the investigation of possible electrical abnormalities particularly important. The cultures were prepared from muscle biopsy specimens which were trypsin dissociated using previously reported techniques (Shain et al. (1977)). Myotubes were chosen for electrophysiological studies on the basis of morphological criteria, such as the appearance of striations, and were studied between three to five weeks after plating.

The results indicate that during classically conditioned flexion, the flexion reflex in the spinal cat, TA alpha motoneurons have an increased probability of firing single action potentials in response to each CS pulse. Support by NSF Grant #8072379.

ELECTROPHYSIOLOGY OF MYOTONIC DYSTROPHY IN MUSCLE. Michael Berdichevsky, S. Prather, and D. E. Broadbelt. Dept. of Neurology, Baylor Col. Medicine, Houston, TX 77030.

Myotonic muscular dystrophy (MMD) is an inherited disease (autosomal dominant) which involves progressive muscular weakness and muscle degeneration. One of the symptoms of the disease is myotonia. Myotonia is a sustained hyperexcitability of muscle cells that is paradoxically triggered by a mild stretch. This condition is thought to be a consequence of altered membrane properties. We have approached the problem of understanding the electrophysiological techniques to investigate the membrane properties of muscle fibers from normal and myotonic patient biopsies which are grown in a primary tissue culture system. Other investigators have attempted unsuccessfully to find morphological abnormalities in cultured MMD muscle. The conclusion drawn is that myotonic disease process is propagated in primary culture, even though it may be modified by the culturing process to some extent. Propagation of the MMD disease process in culture is particularly important because it will permit detailed electrophysiological studies to be carried out to further characterize the abnormality (ie., determination of ionic basis of resting potential decrease) as well as being an excellent system for future biochemical studies of the specific inborn error of metabolism.

SUPPORT: NSF Grant #BNS 77-23845.


Bilateral block of the upper airway in chemothixus altered the neuromuscular activity of several craniofacial muscles. Electromyographic (EMG) activity was recorded from 16 craniofacial muscles in 16 paired animals (13 experimental) during the first 6 months of adaptation. Craniofacial muscles demonstrated two types of EMG patterns: 1) rhythmicity with respiratory pattern; and 2) an increase in spontaneous, uninterrupted discharge (ie., tonic).

The presence of a rhythmic discharge, its sequence within the respiratory cycle and its mode of developing its maximum discharge were assessed by rectifying and averaging the mean voltage for each craniofacial muscle and a primary respiratory muscle. Five craniofacial muscles were rhythmically active in the experimental monkeys and spontaneous, uninterrupted discharge (ie., tonic) was observed in the control animals. The difference between conditioning and sensitization groups. Selection of a motor unit for recording was based upon the discharge behavior during 15 acquisition trials for both conditioning and sensitization. The criteria were such that each unit must have fired at least three to five weeks after plating.

During conditioning, tension in response to the first 10 CS pulses increased an average of 3% compared to pre-acquisition levels. The increase in tension during CS presentations for sensitization animals was 54. Motor unit activity in response to the CS increased an average of 48% during conditioning and 8% for sensitization animals. The difference between conditioning and sensitization responses was more marked for both tension and unit recordings. For individual motor units there was no evidence of the occurrence of multiple discharges in response to any pulse of the CS train. A variation in inter spike intervals of doublets or triplets does not appear to play a role in conditioned facilitation in this preparation.

The results indicate that during classically conditioned facilitation of the flexion reflex in the spinal cat, TA alpha motoneurons have an increased probability of firing single action potentials in response to each CS pulse. Supported by NSF Grant #EYI-77-23845.


Decerebrate cats were made spinal by a T10 cord transection. Classically conditioned flexion reflex facilitation was elicited in the manner of Durkovic (Physiol. and Behav. 14:297, 1975). The conditioned stimulus (CS) was electrical stimulation of the cutaneous saphenous nerve at 10/sec for 1.5 sec. The unconditioned stimulus (US) was electrical stimulation of the cutaneous tibialis anterior (TA) muscle tendon. In addition, single reflexes measured by an isometric tension transducer attached to the tibialis anterior (TA) muscle were measured for a first order histogram. In the control animals, tonicity was induced in several other craniofacial muscles: digastric (72%), anterior temporalis (142%), and lateral pterygoid (10%). The medial pterygoid, zygomaticus and caninus were rhythmically active in 1-2 experimental animals.

Sensitivity was determined by rectifying and integrating EMG activity with automatic resetting of the integrator for 10 trials over 2 hours. The time between the onset of the CS and the peak of the digitized rectified muscle tension was plotted as a function of the latency of the reflex. The latency of the reflex was determined by digital counter for 1000 intervals, displayed on a scope raster, and computed for a first order histogram. In the control animals, sensitivity was seven millionfold. The facilitation reached a maximum of 2.5 millionfold (192), digastric (192), genioglossus (572), lip elevator (267), caninus (373), mentalis (653), and anterior temporalis (533). In the experimental animals, sensitivity was observed in the TA muscle fibers as a baseline increase in tonic tension in these muscles was not changed significantly but tonicity was induced in several other craniofacial muscles: digastric fibers of the tongue (355), superior orbicularis oris (25%), inferior orbicularis oris (32%), genioglossus (57%), mentalis (63%), and anterior temporalis (533). In the control animals, sensitivity was observed in the TA muscle fibers as a baseline increase in tonic tension in these muscles was not changed significantly but tonicity was induced in several other craniofacial muscles: digastric fibers of the tongue (355), superior orbicularis oris (25%), inferior orbicularis oris (32%), genioglossus (57%), mentalis (63%), and anterior temporalis (533). The latypothy, zygomaticus and buccinator were tonically active in 1-2 experimental animals. (Supported by NIH Grant # EYO 2739).


The superior vestibular nucleus (SVN) was explored with HRP loaded microelectrodes in anesthetized, paralyzed cats and rabbits. Stim. electrodes were placed in I11rd nucleus, and on ipsilateral (VI) and contralateral (Vc) vestibular nerves respectively. The above stims. were obtained, HRP was injected. Animals were perfused, frozen sections reacted with CoCl2, and histochemistry. Neurons reconstructed with the aid of a drawing tube.

Neurons responding antidromically to I11rd nucleus stim. are central or dorsal in SVN. They receive monosynaptic EPSPs (13 pairs of stimuli). Some, but not others, receive monosynaptic IPSPs (13 pairs). Many neurons receive both a monosynaptic EPSP and IPSP. Neurons not responding antidromically to I11rd stim. are ventral in SVN. They receive EPSPs and IPSPs. Some of these cells also have collaterals, without collaterals, in the infrahyoid or brachium conjunctivum. These are the relay neurons to I11rd nucleus subserving vestibulo-ocular reflexes. We hypothesize that neurons receiving monosynaptic EPSPs from VI are functionally Type I but should not be as densely packed as neurons with collaterals and without collaterals, across the midline toward the contralateral vestibular nuclei. These neurons receive VI EPSPs. Central SVN neurons also project their axons, without collaterals, to the cerebellum via the brachium pontis. These neurons receive VI EPSPs and Vc-IPSPs. Presumed vestibular commissural neurons are central or ventral in SVN. They have axons with collaterals, across the midline toward the contralateral vestibular nuclei. These neurons receive Vc-EPSPs. Presumed central SN neurons also project their axons, without collaterals, to the cerebellum via the brachium pontis. These neurons receive Vc-EPSPs and Vc-IPSPs. Some neurons, ventral in SVN send their axons ventrally to collateralize in the regular Formation. These neurons receive IPSPs from VI and Vc and are presumed to be functionally Type IV.

To date, no SVN neuron monosynaptically activated from VI has and that of Vc. This surprising finding implies that the SVN components of the vestibulo-ocular reflexes are organized by discrete pathways straight through the nucleus and shifts the excitatory pathways through the SVN.
The intracellular potential of medial gastrocnemius (MG) motoneurons of deeply anesthetized cats was recorded while the MG muscle itself was subjected to continuous band-limited (0-100 Hz) random passive extension-puller connected to its tendon. Stretch amplitude had a Gaussian distribution whose rms value was approximately 100 μm. A time-averaged relationship (cross-correlation function) between the crossed length change and the afferent spike train was computed for several different spindle primaries. This function is proportional to the averaged length signal preceding a spike and is mathematically identical to a shortening-lengthening sequence. Its transform in the frequency domain is suggestive of a system sensitive to rate of change of muscle length primary. MG motoneurons are known to receive synaptic input from a large percentage of MG primary spindle afferents, the motoneuron potential might be expected to reflect the instantaneous change in length of the ensemble of primary afferent discharge. When the transmembrane potential of MG motoneurons was correlated with the random length signal the resulting functions were nearly identical in waveform to the correlation functions obtained from individual afferents using the same input. Given the characteristics of the input signal, the correlation function provides an estimate of the average change in afferent firing probability and in motoneuron membrane potential which would follow an impulse change in MG length, an input roughly equivalent to a tendon tap. Under these particular experimental conditions, therefore, the motoneuron appears to act as a constant gain element whose input-output gain characteristics are largely derived from the frequency characteristics of spindle primary afferent discharge (a contribution from spindle secondaries with similar characteristics can be excluded). We have demonstrated, therefore, that the response of a single motoneuron averaging an ensemble of spindle primary afferent spike trains from its own muscle can be almost indistinguishable from the time average of the response of a single primary afferent.

Supported in part by UPHS Grants: GM 23732, NS 11298, NS 07888 and RR 05675.

CHANGES IN SHORT AND LONG LOOP REFLEXES BEFORE VOLUNTARY MOVEMENTS IN MAN. James A. Mortimer, David D. Webster* and Thomas G. Tatton, Univ. of MN, Minneapolis, MN 55417.

Changes in the magnitudes of short and long latency EMG responses to step increases in load prior to the initiation of a ballistic flexion movement were studied in 8 persons with normal motor function (23-35 yrs). Torques were applied to subjects' forearms through a horizontal support coupled to a pair of DC torque motors. EMG was recorded from bipolar surface electrodes over the biceps and triceps muscles. Subjects were asked to maintain a 90° elbow flexion against a steady 2 Nm load tending to extend the forearm, and instructed to respond to a 2.9 kHz tone by flexing the forearm as quickly as possible. The two indices thus obtained are the frequency characteristics of spindle primary afferent discharge (a contribution from spindle secondaries with similar characteristics can be excluded). We have demonstrated, therefore, that the response of a single motoneuron averaging an ensemble of spindle primary afferent spike trains from its own muscle can be almost indistinguishable from the time average of the response of a single primary afferent.

Supported in part by UPHS Grants: GM 23732, NS 11298, NS 07888 and RR 05675.

FRONTAL LOBE INPUT TO PRIMATE MOTOR CORTEX. Kamel F. Muakkassa* and Peter L. Strick. V.A. Med. Ctr. and Dept. of Neurosurg. and Physiology, SUNY-University at Syracuse, NY. 13210.

There is both controversy and confusion concerning the location of "premotor" areas in the frontal lobe which have direct access to the motor cortex. Our experiments sought to define more clearly the origin of frontal lobe inputs to face, arm and leg areas of the primate motor cortex (area 4). Small single or mixed cell HRP injections were made in the face, arm and leg areas in the motor cortex in 11 monkeys (M. mulatta and fascicularis). In 5 of these animals the injections were placed after face, arm or leg areas were mapped using intracortical microstimulation. HRF injections into area 4 (face, arm or leg) resulted in retrograde labeling of neurons in four spatially separate regions of the frontal lobe. For example, HRF injections into the leg area of motor cortex resulted in labeling of neurons in: 1) the superior limb of the arcuate sulcus (caudal bank), 2) the medial bank of the superior precentral sulcus, 3) the supplementary motor area (SMA), caudally, and 4) the ventral bank of the cingulate sulcus. Each of the four "premotor" areas in the frontal lobe is somatotopically organized. HRF injections into the face area of motor cortex resulted in labeling of neurons in: 1) the inferior limb of the arcuate sulcus (caudal bank), 2) the lateral bank of the inferior precentral sulcus, rostrally, and 4) the ventral bank of the cingulate sulcus, rostrally. HRF injections into the arm area of the motor cortex resulted in labeling of neurons in the four "premotor" areas between the regions labeled following the face and leg area injections. Of the four "premotor" areas the greatest density of labeled neurons was always found in the arcuate and SMA regions. Labeled neurons were also seen in the motor cortex and the four "premotor" areas contralateral to the injection sites. Thus, our results demonstrate four somatotopically organized "premotor" areas in the frontal lobe which project directly to the motor cortex of both hemispheres.

Supported by funds from Neurosurg. Dept. and Veterans Admin.

The EMG activity evoked by sudden perturbations at the ankle differ in normal subjects, cerebral palsy (CP) patients, and patients with adult-onset damage to the CNS. In the normal adult, dorsiflexion produces a stable myotatic reflex in the stretched tibialis anterior (TA) muscle at 110-120 ms, while the anterior tibial (AT) muscle remains electrically silent. Plantarflexion evokes a much more variable response in the stretched TA muscle at 80-120 ms, while the TA group is silent. Thirteen of 14 patients (ages 7 to 27 years) with spastic CP with or without athetosis showed simultaneous responses, at the latency of the myotatic reflex, in the antagonistic AT and TA muscles following forced dorsiflexion. Plantarflexion evoked an abnormally early response (40-45 ms) in the AT muscle. The one patient who did not show this pattern was the only subject who was independent in ambulation and able to participate in sports.

Eight patients (ages 18 to 57 years) were tested who had adult-onset injuries to the CNS, including multiple sclerosis, stroke, incomplete spinal cord transaction, and head trauma. As with the CP patients, clinical evaluations demonstrated hyperreflexia and increased tone in all cases. R. F. Penn and P. E. Burke. (SPON: K. Frank) Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

The EMG muscle in cat acts on the distal phalanx to induce a flexor plantar stretch and pronation and is a major co-contraction of FDL and extensor digitorum longus (EDL) especially in jumps, probably to stabilize the toes.


The neural commands underlying saccadic eye movements have two components: a dynamic component, or pulse (p), which rapidly moves the eye to a new orbital position, and a static component, or step (s), which then holds it there. If these two components are not appropriately matched the eye drifts away from the new fixation point. Such post-saccadic drift is evident in patients with 6th nerve palsies of recent origin (Kommerell et al., Invest. Ophth., *76) and in monkeys recovering from surgical detachment of the extraocular muscles (Optican & Robinson, Soc. Neurosci., *77), but is corrected over a period of days. This has led to the supposition that an appropriate match between the pulse and step components is maintained by an adaptive mechanism which responds to the post-saccadic slip of the retinal image.

We sought to determine whether post-saccadic retinal slip alone was sufficient to elicit this adaptation. Experimental subjects were two rhesus monkeys. Each was seated, with its head fixed, before a translucent screen (subtending 100° x 100°) on which a densely featured image was back-projected. A servomotor-controlled mirror galvanometer in the projector's light path allowed the scene to be moved horizontally under computer control. The animals' eye movements were monitored with the Robinson search coil method and fed into the computer. Immediately after each saccade, the computer caused the scene to drift horizontally, with an exponential time course (40 ms time constant), by an amount equivalent to 45% of the amplitude of the horizontal component of the saccade. In some experiments the scene was made to drift in the same direction as the saccade and, in others, in the opposite direction. After several hours of this optically-imposed post-saccadic slip, both monkeys developed post-saccadic ocular drifts, which were always in the same direction as the imposed visual drift and were evident both in the dark and when the animals viewed a stationary visual scene. The pulse-step mismatch (psm), defined as (p-s)/p100% and normalized to 100%, was used to provide an estimate of the animals' post-saccadic drift: positive values indicate backward drift, negative values, forward drift.

The pulse-step mismatch was abnormal in some animals. Before exposure to the stimulus the mean psm was 0.0 ± 1.9% (N=48). After 8 hours of exposure to opposite-direction slips the mean psm was 5.5 ± 2.3% (N=50, range 0.6 to 10.3%) and the mean psm following stimulation was -10.6 ± 2.4% (N=66, range -2.8 to -23.1%). The time constant of the ocular drift was similar in both situations, with a mean of 69 ± 25 ms (N=47).

Eye movements in squirrel monkeys were recorded, in darkness, before and after unilateral HC inactivation, obtained by a plug­ging procedure (Money & Scott, 1962). Monkeys were rotated sinu­soidally in the HC plane, within a bandwidth of .01-0.4 Hz, and at constant amplitude (120°/sec). At 2 Hz, intensity series (40-360°/sec) were presented at .02 and .2 Hz.

In normal monkeys, VOR gain is relatively flat and averages 1.0 over the recorded bandwidth. Phase lead (re velocity) is near 0° from 4° to .1 Hz, increasing to near 45° at .01 Hz. The effective time constant (T_e) of 17 sec is 2-3 times greater than that of peripheral Vestibular afferents (Goldberg and Fernand­ez, 1971). Intensity series data display gain and phase linear­ity at .2 Hz. Phase lead at .02 Hz decreases from ~25° at 40°/sec to ~12° at 120°/sec, with little further change at higher amplit­ude. No gain change accompanies this effect.

Recordings made within 2 days of HC plugging indicate that VOR gain is near 500%. Gain and phase linearity is normal. Spontaneous nystagmus (SN), with slow phase ~10°/sec to the plugged side, is always present.

During the month following HC plug, three significant modi­fications of the VOR are observed: a) generalized gain recovery (to ~75% of normal); b) increased low frequency phase lead (~0.01 Hz), usually accompanied by gain reduction, such that T_e is reduced to near that of peripheral afferents; and c) SN is grossly modified. Animals recovering from HC plug in a normal cage environment display most of these modified modifications during the first week. If either vision or head movement is prevented during this time, gain remains virtually fixed. However, reduction in T_e is always observed. SN is still present and is often enhanced.

Recordings from peripheral afferents of HC-plugged monkeys are consistent with the notion that the canal plug effectively elim­inates canal function, leaving tonic vestibular input intact. (Supported by NHI Grant GM-07281).


The control of head posture is important for stabilizing gaze and whole body posture relative to gravity. For this, vestibular reflexes which are dependent upon stimulation of the verti­cal semicircular canals and otolith organs play a major role. In order to quantitatively assess the vestibular contributions, one can define the relation between input accelerations and the neck muscle (ENG) responses in restrained animals. Recently, Anderson and Pappas (Soc. Neurosci. Abst., 1978) have reported this, and in particular to characterize the overall reflex dynamics in alert, restrained cats during sinusoidal roll and pitch rotations. The results indicated that both vertical canal and otolith contributions make significant contributions to the motor responses and b) both "direct" and "indirect" pathways must be involved. Regarding the latter, it should be noted that after chronically sec­tioning the descending MLF (2-8 day prior to recording), it has been found that there is a profound disturbance of the reflex dynamics: For low frequency (less than 0.15 Hz) pitch rotations the phase of the muscle response regarding angular acceleration shows much less of a lag, approaching that of the canal affer­ents themselves (Anderson and Pappas, European Neuro. Soc. Abst., 1979). Compared to normal, this indicates that a response to the otolith inputs and to a neural integration of the canal in­puts was reduced.

To account for this we postulate that the midbrain reticular formation, i.e., the region of the interstitial nucleus of Cajal (INC) and the rostral interstitial nucleus of the MLF, is neces­sary for the proper convergence of the horizontal canalthal inputs and for the neural integration of vertical canal inputs (Pola and Robinson, J. Neurophysiol. 41:245-259, 1978). The processed signal could possibly be carried in the ascending medial or MVST pathways in the MLF. As a first step in this, we made bilateral, electrolytic lesions in the region of INC in 4 cats. 2-8 days thereafter we recorded the ENG responses to head movements, the abdovestus cervicis muscle when that cats (alert, restrained, and blindfolded) were subjected to sinusoidal sinusoidal and pitch rotations. The results from all the animals 2-8 days after lesions were similar to that of the MLF lesioned cats: For low frequency the ENG showed much less of a lag, e.g., 80-110 deg at 0.15 Hz instead of 130°/sec. In the normal cats and higher frequencies (1.0-3.0 Hz), the phases were approximately normal. These data thus do provide some support for the proposed role of the mid­brain nuclei. To further test this we are attempting to selectively destroy only the neuron populations, leaving fibers in passage undisturbed, by using the neurotoxic agent, kainic acid.
INTERACTION OF VESTIBULAR AND NECK REFLEXES IN CONTROL OF NECK MUSCLE ACTIVITY. B. M. Peterson, J. H. Fuller, G. Bilotto and V. J. Wilson. The Rockefeller University, New York, N.Y. 10021.

Reflex stabilization of the head during angular rotations in the horizontal plane was studied in precocial decerebrate cats with their heads tilted 25° forward from the stereotaxic plane in a holder that allowed rotation of the head about a vertical axis passing through the C2 vertebra. Horizontal rotation or torque about this axis was measured together with EMG activity of neck muscles that produce horizontal head movements. During such movements, the NNR and VCR were elicited alone by whole body rotation with the head fixed, loads applied to the moving head. In our preparation the NNR head rotation was always less than half that of the turntable, and the NNR and VCR head rotation were 162° behind acceleration at 0.2 Hz. Although the amplitude of EMG activity of neck muscles that produce horizontal head movements was constant from day to day except for a slight slowing towards the end of the experiment. No evidence of a division into two groups of fast and slow units as observed by Close (1967) in rat soleus was observed, 32 of the 45 units (i.e. about 70%) had times to peak of less than 20 msec, the upper limit of the faster group of units seen by Close in rat soleus.


Supported by grants from the Muscular Dystrophy Group of Great Britain and the Muscular Dystrophy Association of Canada. R. P. was the recipient of a Canadian MRC Visiting Scientist Award and was on leave of absence from Dept. Physiol. Univ. of Ottawa, Ontario, Canada.


When a standing quadruped makes a limb movement, a change in posture occurs which permits the execution of the movement without loss of balance. Previous work has shown that when the limb movement is triggered by a tactile/proprrioceptive stimulus on a leg, the weight supported by the leg decreases while the contralateral hindlimb increases. When the weight supported by both hindlimbs is increased during a paired movement, the change in posture is much greater than the movement itself. These observations suggest that this pattern is organized at bulbo-spinal levels as are those for locomotion.

Five cats were used in these experiments. The animals were trained to stand quietly with their paws on platforms which measured vertical force. Moveable platforms, right and left, were used to elicit a stepping reaction by a forelimb. The animals were also conditioned to lift either forelimb on command using a preparatory tone followed by a beeping tone and flashing light to trigger the movement. The pattern of postural adjustment during this type of movement was different from that during placing: weight transfer was primarily between the forelimbs, although there was sometimes a symmetrical increase in weight under the hindlimbs. Placement movements elicited after training the cats to lift on command tended to be less diagonal and approached the pattern observed for conditioned movements.

In 3 cats the motor cortex was electrically stimulated shortly after the signal eliciting a movement was presented. Cortical points in the forelimb area were used. The pattern of postural support accompanying the stimulation-induced movement was less diagonal when the lifted limb was the one that was to be used in the conditioned movement than when the stimulation-elicited lift involved the contralateral limb. This response asymmetry disappeared during control sessions in which the cat was required only to stand quietly.

These experiments suggest that postural commands accompanying movement target final positions not only for the cat's center of gravity, but also to specific sites on the vertical column and suggest that a "tuning" of the output circuits precedes overt movement. In addition, the use of conditioned movements in a postural context provides an interesting approach to the study of mechanisms by which new patterns of postural support are acquired.

Supported by grants ET 02249, ET 00100 and NS 02619.
Adaptation of muscle spindles to stretch is thought to be largely due to a viscoelastic relaxation of the intrafusal muscle fibers, and it has been shown that dynamic bag fibers (bag) in particular do exhibit such viscoelastic relaxation following a quick stretch. This is a mechanical adaptation which will result in a phase lead in the response of the receptor to sinusoidal stretches, a fact which provides an opportunity to determine whether the mechanical adaptation is quantitatively adequate to account for receptor adaptation. Measurements of the phase lead of responses to small amplitude sinusoidal stretches were made in isolated cat tenenimus spindles. Strain (or the change in length per unit length) across the primary sensory area of bag fibers was plotted as a function of time along with the primary afferent discharge rate for applied stretch frequencies from 0.05 to 2 Hz. The results suggested that only a portion of the phase lead exhibited by the primary afferent can be accounted for by muscle viscoelasticity. The maximum phase lead was 45° in the neighborhood of 0.2 Hz where it is still approximately 30° less than the phase lead exhibited by the primary output. Sensory strain is in phase with the applied stretch, whereas the primary ending output has a phase lead of 90° at 25°C. Therefore if afferent receptors are to be quantitatively accounted for by mechanical factors, these must be associated with structures within the sensory area and not with the viscoelasticity of intrafusal muscle poles.

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RESPONSES OF ASCENDING TRACT OF DIETER'S (ATD) NEURONS TO NATURAL AND ELECTRICAL STIMULI. V. Beilina and S.M. Hignite, Albert Einstein College of Medicine, Bronx, New York, 1046l.

The ventrolateral vestibular nucleus (VLV) was explored with intra- and extracellular microelectrodes in anesthetized, paralyzed cats. Stimulating electrodes were placed in the IIIrd nucleus and on the ipsi-(VI) and contralateral (Vc) vestibular nerves. The MLF and brachium conjunctivum (BC) were acutely severed in the pontine tegmentum. Neurons in the VLV, antidromically activated by IIIrd nucleus stimulation received monosynaptic EPSPs from VI and disynaptic IPSPs from Vc. Neurons with the above profile of responses were injected with HRP and subsequently were invariably found to lie in the VLV.

Antidromic activation of VLV neurons was signalled by an antidromic field potential in VLV. This potential was abolished by lesions 1 mm wide between the MLF and BC in the pontine tegmentum indicating the course of the ATD.

In a second set of experiments decerebrate cats were rotated about a vertical axis in the plane of the horizontal semicircular canal. Stimulating electrodes were implanted as above. VLV neurons were isolated extracellularly and identified as ATD neurons by their antidromic responses to IIIrd nucleus and orthodromic (monosynaptic) responses to VI stimulation. Responses of these neurons are in phase with table (head) velocity in the range of ±30°/sec. at 0.4 - 0.65 Hz increasing to ipsilateral and decreasing to contralateral rotation (Type 1 responses). This head velocity signal should be transmitted directly to medial rectus motoneurons and consequently to ATD and unipolar horizontal vestibulo-ocular reflex in intact cats. The remaining reflex following bilateral MLF lesions may be accounted for, in part, by the head velocity signal carried by the intact ATD.


Last year we presented evidence that reflexively-induced changes in isometric force at a given rate of stretch after extrafusal threshold was reached could be attributed to beta fibers. To better appreciate the nature of beta effects, an understanding of the interaction of muscle tone and stretch is necessary. The purpose of this present study was to identify possible beta influence on spindle receptor discharge during constant velocity stretch and isometric shortening and to correlate these results with those isometric responses presumed to be of beta origin.

We recorded from deep surasse spindle receptor afferents in isolated dorsal root filaments of 13 decerebrate cats. Decerebrates were used so that variations in initial muscle force could be induced in a consistent manner via crossed extensor stimulations (CES). The receptor-bearing muscles were subjected to stretch with and without CES, and to reflexively-induced isometric shortening of the muscle stretcher providing an electronically simulated load.

Of the 60 units examined with ramp stretch, 36 showed additional increases in discharge during CES. Response patterns could be discerned: 1) an increase in discharge during the ramp (11/36) termed dynamic type, 2) an increase in discharge during the pre- and post-ramp periods only (16/36) termed static type and 3) a combination of 1 and 2 (9/36), static-dynamic type. Of these 36 units showed a large rate acceleration during stretch not paralleling the unstimulated responses. Since gamma fibers are relatively unresponsive to stretch, these units seemed to be the most likely candidates for beta activity. Supportive evidence for a beta contribution was provided in that 7 of these 10 units showed isometric acceleration.

Isotonic shortening was studied in 28 units. The isometric responses of these units are similar to those seen during a quick stretch. This isometrically unresponsive behavior during shortening; 6 of the 7 units that decreased their rate with increases in isometric force also showed a diminution in rate (=9.38 ips/mm, 1.1-3.24) during shortening. The remaining 2) a limited decline or even an acceleration in rate during shortening would be anticipated.

It appears that identification of beta fibers may be possible by discerning a constellation of response patterns during iso- metric and dynamic states.
MOTOR SYSTEMS

1292 THE ACTIVITY OF RETICULO-SPIRAL CELLS DURING Locomotion in Cats. S. Rossignol and J. Parent. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal Montréal, Québec, Canada, H3C 3T8.

Reticulo-spinal cells were recorded extracellularly at the cervical and posterior levels during locomotion of collicullarly decerebrated cats. Cells were identified by high frequency antidromic stimulation through a thin silver strip in- serted under the skin. EMG of the quadriceps and the triceps Radialis were recorded on both sides. The cervical vertebrae were solidly fixed and all 4 limbs were allowed to move. A good number of cells were kept for several hundred cycles allowing to record their activity at different walking speeds (walk and trot) as well as during manual blockade of one or more limbs. Of the 31 cells recorded, 27 (87%) were modulated while 4 (13%) remained silent or were silenced during locomotion. Cells discharging in distinct bursts showed a positive correlation between the burst duration and the duration of the step cycle at various speeds, indicating a relation to a component of the step cycle which changes with speed namely the stance phase, the duration phase being considered practically constant at various speeds. The peak firing of different cells in relation to the step cycle was found to vary. The stability of the firing pattern of a given cell was tested by varying several walking speed which in a number of cases changed the phase relationship between fore- and hindlimbs as well as by blocking the limb. The H-reflex was recorded carefully analyzed by computer. It was possible to show, when triggering the analysis on the EMG of one forelimb, that the firing pattern was invariant when the forelimbs were walked alone or with the hindlimb or the hindlimb and even when the coupling changed between fore- and hindlimbs. This strong dependence of the cell discharge on the fore- and hindlimb could be set out by the cells more clearly when recording the forelimbs which stopped the firing although the hindlimbs continued walking. At times when the animal initiated a period of locomotion with both hindlimbs, the cells started firing later when the forelimbs also walked. Although cells could discharge with only the forelimbs walking, the frequency of discharge often changed when limbs walked. The strong dependence on forelimbs of the discharge of reticulo-spiral cells with axons identified in the lumbar cord might suggest that they play a role in the coordination of fore- and hindlimbs during locomotion.

(Supported by a group grant of the MRC. J.P. received a fellowship from the Ministry of Education, Quebec)

1293 Absence of Force-Feedback Contributions to the Stretch Reflex of the Decerebrate Cat. W.E. Wymer, Northwestern University Medical School, Chicago, Ill. and F. Haase, SUNY-Upstate, Syracuse, N.Y.

The reflex force evoked by stretch of the soleus muscle in the decerebrate cat is the result of a spinal reflex, and is largely independent of stretch velocity. It has been suggested that this spring-like behaviour arises from the combined effects of stiffnesses which are independently evoked by afferents derived largely from tendon organ and spindle receptor input respectively. We examined the contributions of force-feedback to stretch reflex action with the help of Dantrolene sodium, an agent known to reduce contractile force via excitation-contraction decoupling. Experiments were performed in the soleus muscle of 12 decerebrate cats. The calcaneal insertion was severed, and attached to a servo-regulated muscle stretcher. EMG signals were recorded differentially using fine wires implanted in the muscle, and the EMG was rectified and filtered with 25 msec. time constant. Force and length were also measured.

In each animal, a series of stretches of constant amplitude and velocity were used to record force and EMG responses, followed later by a period of inhibition. Initial force was recorded, followed by an increase induced via the crossed extensor reflex. The animal was then given successive 1.0 mg doses of Dantrolene intravenously, until peak tension was reduced by at least 30%. The stretch reflex responses were then reexamined, again using crossed extensor stimulation to vary initial force levels. In every case, reflex stiffness was markedly reduced, yet there was no significant increase in the EMG incremental response. Moreover, the lack of EMG change was not a result of Dantrolene effects on muscle spindle. The monitored response of single spindle receptor afferents showed no significant alteration until much larger cumulative doses of Dantrolene were achieved (typically greater than 5mg/kg). We fixed force-feedback to contribute to the spring-like behaviour, a reduction in contractile force should have been followed by an increase in EMG. Since no such increase occurred, it is concluded that force-feedback contributions to the stretch reflex are insignificant in this preparation.


1294 EFFECT OF TOPICAL ANESTHESIA TO VARIOUS SKIN AREAS ON THE H-REFLEX, AND TRIGGERING OF FACILITATION. M. A. Habib, P. C. Wiggs, D. B. de Luca (SPON: J.V. Basamajian) Dept. of Health Sciences, Sargent College, Boston University, Boston, Ma.

The H-reflex was recorded from a carefully analyzed by computer it was possible to show, when triggering the analysis on the EMG of one forelimb, that the firing pattern was invariant when the forelimbs were walked alone or with the hindlimb or the hindlimb and even when the coupling changed between fore- and hindlimbs. This strong dependence of the cell discharge on the fore- and hindlimb could be set out by the cells more clearly when recording the forelimbs which stopped the firing although the hindlimbs continued walking. At times when the animal initiated a period of locomotion with both hindlimbs, the cells started firing later when the forelimbs also walked. Although cells could discharge with only the forelimbs walking, the frequency of discharge often changed when limbs walked. The strong dependence on forelimbs of the discharge of reticulo-spiral cells with axons identified in the lumbar cord might suggest that they play a role in the coordination of fore- and hindlimbs during locomotion.

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Rhesus monkeys were conditioned with light signals to perform four randomly selected tasks with the hindfeet. Force was exerted bilaterally on fixed bars with attached strain gauges. The tasks were: (1) to exert a strong plantarflexor force, hold it a random time, on cue exert a strong, brief, rapid dorsiflexor force and return to rest (rapid-relax); (2) to do the converse; (3) to plantarflex into a small force window, continuing during the hold period (tonic cells, 20%); (4) those with strong activity occurring preceding the achievement of the required force (phasic cells, 22%); (5) dorsoflexor phasic cells, 22%; (6) dorsiflexor tonic cells, 20%; (7) cells whose major activity occurred related to the time of initiation of and maintenance of small plantarflexion hold, 8%; and (6) cells whose greatest firing occurred during the return to rest at the end of each task, 3X.

Average resting discharge frequency (ADF) was calculated for the 500 msec. preceding the presentation of the first signal of each task. Among plantarflexor cells, most phasic cells had low ADFs (56% less than 13/sec.) while only a few of the tonic cells had low ADFs (21%). There were higher ADFs for the majority of dorsiflexor cells (64% tonic, 73% phasic). Phasic cells (69% plantarflexor and 83% dorsiflexor) fired faster in the initiation of the rapid-relax action than in the initiation of the hold action. During the 200 msec. post-Cn discharges continued at a faster rate (greater than 10% difference) after the hold versus the rapid-relax action (64% of all cells). Less than 30% of the cells showed greater activity with a strong hold force than with a small hold force.
COMPARISON OF MORPHOLOGICAL FEATURES OF FAST AND SLOW PT CELLS REVEALED BY INTRACELLULAR PRESSURE INJECTION OF HRP. H. Sakai* and C.D. Moody. (Spon: E. Eldred) UCLA Medical Center, Los Angeles, CA. 90024.

Recordings were obtained from the motor cortex of 14 awake cats using the HRP-injected, filled electrode technique. In this preparation, 19 PT cells were analyzed. Eight of the cells were also injected with HRP-CMP. Intracellular injections were made with a pressure of 80-100 psi for 0.5-2 sec to facilitate the responson of each cell to antidromic stimulation. The cells were recorded by core biopsy. The pyramidal tract was stimulated ipsilaterally at the level of the facial nucleus (P.S. S.O.: L. 1-19) utilizing monophasic (two, 0.5-msec pulses of 0.33 msec duration delivered every sec). Distance from the recording area to the tract was determined histologically. The stimulus strength used to find the locus of antidiromic stimulation was between 4 and 5 cm. Cells which were activated antidromically with the latencies shorter than 2.5 msec were classified as fast PT cells. Cells with velocities of at least 20m/sec. Cells activated with latencies longer than 2.5 msec were classified as slow PT cells (n=6). Two cells with latencies between 2.0 and 2.5 msec could not be classified into either group.

The following observations were made:
1) The diameters of axons measured at a level 10-23m beyond the axon hillock were larger in the fast conducting PT cells (correlation coefficient, r=0.738 between axon diameter and conduction velocity).
2) All PT cells with somas recovered (n=17) were pyramidal cells located in layer V. Fast PT cells had larger somas (30-70um) than did slow PT cells (15-30um). This confirms the result obtained by Ait. (Brain Res. 1906) obtained by methyl blue staining.
3) The lateral extent of the dendritic field within layer V was greater in fast PT cells as compared to slow PT cells.
4) Slow PT cells had high densities of spines on dendrites in layer III in agreement with Labelle and Deschenes (Brain Res. 1979). The number of spines on dendrites that one-third of the fast PT cells injected with HRP without CMP did also. Thus the density of spines in layer III was not as reliable an index for separating fast and slow PT cells as soma size and axon diameter.
5) Electron microscopic examination done collaboratively with C. Bitak, U.C. Irvine, showed dense amounts of HRP reaction product within neurons without spread of HRP into the extra-axial space. Although the internal structures were obscured by reaction product, the membranes and synaptic junctions were well preserved. (Supp. by HD 05958 & E. Gruen)


The effects of single precentral microstimuli on activity of 21 identified forelimb muscles were documented in monkeys making active wrist movements. Stimulus-triggered averages (StTA's) of rectified EMG activity were obtained. No facilitation of suppression of muscle activity produced by intracortical stimuli (2.2 ms, biphasic), using stimulus intensities too low to evoke overt responses (up to 100µa), may be due to the temporal summation (4.5/5sec). When applied at cortical sites of cells producing post-spike facilitation in spike-triggered averages (EDC activity), no facilitation was observed. The predominant effect on R1 was a facilitation which paralleled the pattern of post-spike facilitation. Additional facilitation was achieved by applying 0.03 msec pulses of 0.5-0.6 volts delivered every second. Distance from the recording area to the cortex was determined histologically. The stimulus strength used to find the locus of antidiromic stimulation was between 15 and 50 sec. Cells which were activated antidromically with the latencies shorter than 2.5 msec were classified as fast PT cells. Cells with velocities of at least 20m/sec were classified as slow PT cells (n=6). Two cells with latencies between 2.0 and 2.5 msec could not be classified into either group.

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VOLUNTARY MOVEMENT AND EXCITABILITY OF HUMAN EYELID REFLEXES. Jerome N. Sanes* and James R. Ison. Dept. Psychology, University of California, Santa Cruz.

These findings (1) substantiate the claim that reflex excitability is a sensitive and specific indicator of facial and other supraspinal influences on motoneuronal pools before and after voluntary movement; (2) further elucidate characteristics of reflex recovery from suppression of muscle activity; and (3) indicate that reflex facilitation or depression of a more generalized motor readiness system. Reflex suppression was the predominant effect on R2, even though its final common path was not identifiable with increased R1 facilitation. The suppression of this net inhibition must lie in the longer associative pathways for R2 elaboration. Thus, these data illustrate net facilitative and inhibitory effects by the same behavior on reflexes with identical afferent and efferent limbs but distinctive central pathways.


Two Rhesus monkeys were trained to perform wrist movements guided by a continuous pursuit tracking display. The target, and therefore the wrist, moved in a hold/ramp/hold track. Parameters of the target's motion were selected from preset values, directly relevant to the detected subject's facilitation of stimulus, and the magnitude of suppression of muscle activity produced by intracortical stimuli (2.2 ms, biphasic), using stimulus intensities too weak to evoke overt responses (up to 100µa), may be due to the temporal summation (4.5/5sec). When applied at cortical sites of cells producing post-spike facilitation in spike-triggered averages (EDC activity), no facilitation was observed. The predominant effect on R1 was a facilitation which paralleled the pattern of post-spike facilitation. Additional facilitation was achieved by applying 0.03 msec pulses of 0.5-0.6 volts delivered every second. Distance from the recording area to the cortex was determined histologically. The stimulus strength used to find the locus of antidiromic stimulation was between 15 and 50 sec. Cells which were activated antidromically with the latencies shorter than 2.5 msec were classified as fast PT cells. Cells with velocities of at least 20m/sec were classified as slow PT cells (n=6). Two cells with latencies between 2.0 and 2.5 msec could not be classified into either group.

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5) Electron microscopic examination done collaboratively with C. Bitak, U.C. Irvine, showed dense amounts of HRP reaction product within neurons without spread of HRP into the extra-axial space. Although the internal structures were obscured by reaction product, the membranes and synaptic junctions were well preserved. (Supp. by HD 05958 & E. Gruen)
A MAPPING OF BRAINSTEM NEURONS PROJECTING TO THE SPINAL CORD IN THE GOLDEN HAMSTER. Randall C. Shults and James D. Rose. Dept. Psychol., Univ. of Wyoming, Laramie, WY 82071.

As part of an investigation into the brainstem control of estrous reflexes in the female hamster, brainstem neurons projecting to the spinal cord were identified with the horseradish peroxidase (HRP) technique. A 50% solution of estrous reflexes in the female hamster, brainstem neurons projecting to the spinal cord were identified with the technique of Mesulam (J. Histochem. Cytochem., 1978). Relatively more neurons, especially ipsilaterally, were observed in the reticular formation, bilaterally. The number of cells labeled in these regions diminished progressively as well as with sacrifice of HRP injection. Conventional microelectrodes were used to explore the area with the precentral cortex for cells that were temporally correlated to the task. The cell's sensory field, response to torque pulses, and whether or not the cell projected into the pyramidal tract was determined prior to intracortical microstimulation (ICMS) through the recording electrode. Trains of 17 stimuli (.2 ms biphasic pulses at 400 Hz and current levels to 20 µA) were delivered at specific times during the task when the motorneuron pools to the forearm muscles were involved in different phases of the movement. By applying the ICMS under the same load and movement conditions repeatable EMG responses in forearm flexors and extensors were obtained. ICMS produced a larger excitation when a movement was being initiated against a constant load than when the muscle had been opposing the same load for approximately 1 sec. The time delay between the start of the ICMS train and excitation or inhibition of forearm muscles was as short as 11 ms. Quite often excitation preceding EMG activity was recorded as shown in Fig. 1A. This was not due to cortical reverberating circuits because the EMG activity would end abruptly 11 ms after the last stimulus pulse of the train. Areas of inhibition of a given muscle have been observed as close as 100 µm to areas of excitation of the same muscle. EMG patterns produced by ICMS have ranged from inhibition of a single muscle (Fig. 1B), inhibition of contralateral muscles, and excitation of synergists, inhibition and excitation of antagonist, to excitation of single muscles. There is some indication that excitatory regions to a specific muscle are bordered by inhibitory regions to the same muscle. Experiments on additional animals are required to further explore this finding.

![Fig. 1. Rectified and averaged (32 trials) EMG responses in flexor carpi radialis (FCR) to ICMS in precentral cortex. The calibration bars below the records indicate the time duration of stimulation. (A) Ramp increase in EMG activity of FCR during movement. (B) Inhibition of FCR at a site 4 mm from A.](image-url)


Behavioral and photographic techniques were used to determine the movement correlates of discharge in medullary RF units recorded in unrestrained cats. These cells could be divided into two major classes on the basis of the behavioral correlates of their discharge: cells with laterally asymmetrical movement relations, and cells with laterally symmetrical movement relations. Fifty-four percent of encountered cells (n=21) had laterally asymmetrical movement relations while 38% (n=15) had laterally symmetrical movement relations. The remaining cells (n=3, 8% of total) did not relate to any specific motor behavior.

The largest subgroup (n=16) of cells with laterally asymmetrical movement relations was comprised of cells related to movements of the head or vertebral column in the horizontal plane. Of these, 12 responded when the head was moved passively. In all of these cells, increased discharge was related to passive head movement to the ipsilateral side. This asymmetry is highly significant (p<0.005). Cells responding to passive head movement to the contralateral side did not discharge when the same movement was made spontaneously by the cat. Instead these cells discharged only when the cat turned to the ipsilateral side.

Cells without lateralized behavioral relations (n=15) could be divided into those related to head or spinal movements (n=12) and those related to other movements. Of those related to spinal movements, six discharged in relation to active forward movements of the head when it was in a lowered position, a seventh cell discharged only during active ventral movements of the lumbar spine, and an eighth discharged during fixation of the lumbar region. Four responded during spontaneous upward movements of the neck. Three of the four cells that responded to active head movement responded only when the head was moved in a direction opposite to the optimal spontaneous movement.

The behavioral relations shown by these cells indicate that many may have a pattern of synaptic relationships that coordinates excitation and inhibition of several motorneuron pools involved in producing ipsilateral somatic movements. The present results suggest that the cellular mechanism underlying the classic "terminal response" of ipsilateral curvature of the spinal column after electrical stimulation of the RF.

(Supported by the Veterans Administration and N.I.H. grant NS14610)

Myopotentials from two ankle extensors, the soleus (SOL) and the lateral gastrocnemius (LG) were recorded from chronic spinal cats transsected at T13 either at 2 or 12 weeks (W) of age. Four cats in each group were exercised daily on a motorized treadmill for 10-20 min, and testing was completed 2 weeks post-surgery. With the forelimbs and trunk suspended above the treadmill, all cats fully supported the weight of their hindquarters during stepping. Most cats periodically used their hindlimbs in a step pattern during quadrupedal standing and overground locomotion. Muscles were implanted with electrode wires under sterile operating conditions as described by Betts et al, Brain Res. 117:529, 1976.

The electromyography (EMG) was synchronized with the cat's movement by a video system and recorded on FM tape for subsequent integration and period analyses by minicomputer (Smith et al, J. Neuropyschol. 40:503, 1977).

During treadmill locomotion, the SOL was recruited at all speeds (0.13 to 0.89 m/s), which elicited the entire range of gaits from a slow walk to a slow gallop. The peak amplitude of the rectified-averaged (RA) EMG, which in normal adult cats is correlated with peak tendon forces measured in vivo (Smith et al, J. Neurophysiol. 41:1203, 1978), increased about 20% for SOL and about 300% for LG over the range of speeds tested. During unassisted standing and stepping overground, the SOL was recruited alone, and the RA-EMG was similar to that recorded during slow treadmill stepping.

When the cats were held vertically with hind legs pendant, air stepping was recorded at 3-5 c/s, and the SOL was active generally without the LG. Clonus, elicited by tendon taps or during slow treadmill stepping and standing, ranged from 10-15 c/s and was predominant in the SOL.

During rapid and alternate ankle movements of 10-14 c/s, produced by sticking tape to the plantar pads, the LG alone was active, as is the pattern for normal adult cats (Smith et al, J. Neurophysiol. 41:1203, 1978), only in those cats in which the contraction time (CT) of the SOL, measured at sacrifice, was >50 ms. However, in those cats which did not exhibit LG activity, the SOL was active generally without the LG during all speeds tested.

Discrete electrolytic lesions were placed in the dentate and interpositus cerebellar nuclei and the resulting anterogradely degenerated fibers were stained with the Witanen technique. Dorsal parts of the dentate project to ventral parts of RNpc and VL. Rostroventral dentate to lateral hindlimb areas in RNpc and VL. There is a small projection from the crus II to the lateral gastrocnemius (LG) and about 300% for LG over the range of speeds tested. In some cats, recruitment of a "fast" SOL would be preceded by recruitment of the LG, and SOL were recruited simultaneously. All weight-bearing 2W bursts were preceded by recruitment of the LG, and the RA-EMG was similar to that recorded during stepping. Most cats periodically used their hindlimbs in a step pattern during quadrupedal standing and overground locomotion. Muscle EMG were implanted with electrode wires under sterile operating conditions as described by Betts et al, Brain Res. 117:529, 1976.

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Macaque monkeys, trained to make repetitive saccades, were used to evaluate the method. The procedure involved the injection of a small amount of 2-deoxy sugars (2DG) and measuring the brain glucose usage. The monkeys were trained to make repetitive saccades and to make them at different speeds. The 2DG was injected into the lateral ventricle and the brain glucose usage was measured. The results showed that the method worked well and that the monkeys were able to make repetitive saccades at different speeds. The method was effective in identifying the regions of the brain involved in the generation of eye movements. Monkeys were taught to follow a saccade target presented on a large screen oscilloscope. A saccade was defined as a smooth pursuit movement and a fixation saccade was defined as a movement to a new position, requiring another acquisition saccade and fixation interval. Since trials were presented without an interval between saccades, the number of saccades during the fixation period, the number of spontaneous saccades was minimized. For example, one monkey made over 2000 saccades and over 700 fixation periods, 9 periods in the second fixation period. The number of saccades with other vectors occurring during this period was negligible.

After extensive training, the unanesthetized monkey (with indwelling intravenous catheters) was placed in the experimental chamber, the head restrained, and the tracking task begun. Horizontal and vertical eye position signals (Fuchs and Robinson, 1960) were sampled at a rate of 250/sec throughout the 6 minute session and stored on a 9-track digital magnetic tape. Thus, it was possible to reconstruct the vector of each eye movement that occurred during the experimental session. If satisfactory performance continued (after an initial injection of physiological saline, a rapid injection of (14C)-2-deoxyglucose, 70 µCi/kg in 1.5 ml of saline was given. At the end of the 45 minute tracking period, the animal was anesthetized with Nembutal and perfused intracardially with 3.3% phosphate buffered formalin at pH 7.4. The brain was removed, bloomed, and slowly submerged in Freon 22, chilled by an acetone/dry ice mixture.

Autoregions revealed increased levels of metabolic activity in extensor oculomotor muscles and in the brain stem regions of the lamprey, abducens and abduces nuclei; pontine oculomotor regions; and the cerebellum. Sections from other oculomotor areas are currently being processed. Sections labelled with the 2DG in the labeled nuclei showed specific regions of discrete folia showing increases in activity. The midline vermis region showed generalized increases in label, somewhat denser in the ventral and the dorsoventral borders of lobules V, VI, and VIIIB. Laterally, discrete patches of label were observed in lobules IVC and VIIB of the vermis, in the middle and paramedian lobules and in Crus Ila. All folia of the flocculonodular examined showed dramatic, but uniform, increases in metabolic activity.

In combination with appropriate behavioral procedures, the (14C)-2-deoxyglucose technique (Fuchs et al, 1977) appears to be suitable for investigations of the oculomotor system. (Supported by NIH Grants EY 01189 and EY 02293).
Merton, in his classic work of 1953 on servo-control of move­
ment (The Optimizing Matrix, p. 297-299). He stated that the po­
sition of a limb may be controlled by a negative feedback, servo­loop mechanism (a length servo) involving primarily the short­er reflex. He also noted that the long limb arc around the servo loop would have to be compensated for by derivative, or rate, feedback if oscillations of these limbs were not to occur. Further, he pointed out that the muscles lima­
dles are well adapted to provide that compensation because of their very marked response to any rate of change of muscle stretch. The question of whether the rate feedback normally does compensate for the time delay in the servo loop has been con­
sidered since by many different workers. However, it appears that the question is still unanswered.

A measure of the time lag in the servo loop(s) can be ob­
tained for postural hand tremor by performing cross-coherence analysis of the demodulated extensor 350 and the hand motion. Using this procedure, the delay (in msec) between when the peak upward position of the oscillating hand occurred and the time later that the maximum (peak) of the amplitude modulation of the extensor 350 occurred was calculated. This time difference (de­
signated Δt) was determined for nine normal human subjects. For five of the nine subjects, the Δt increased from control values of between 30 and 60 msec for control tremors with rms displacements of between 30,000 to 35,000 micra. However, for the sixth subject, the Δt increased from control values of about 30,000 to 15,000 micra. Even so, the Δt values increased very little above control levels of about 30 msec. For the seven remaining subjects, however, the Δt values changed very little as the subject maintained the unsupported hand in a horizontal position. For the 56 additional seconds analyzed of this subject, the Δt values increased very little above control values of about 30 msec. For the remaining subject, the Δt values decreased very little from control levels of about 10,000 to 30,000 micra. The Δt values increased very little above control values of about 30 micra. For six of the nine subjects, the dis­
placement amplitude and postural hand error also appeared to depend on the particular hand pathway(s) (Sup­
ported in part by USPHS Grant HD 14730.)

Five preoperatively trained dorsal rhizotomized (DR) monkeys performed a visually cued reaching task within two months of surgery (D. Berman, et al., 1978). Testing was performed in the dark and the visual cue was a light as small as 300 by 300 by 300 micra for hand tremors with rms displacements of between 10,000 to 20,000 micra. One subject's tremor increased very little in displacement amplitude over two different 60-min periods that the subject maintained the unsupported hand in a horizontal position. For the 56 additional seconds analyzed of this subject, the Δt values increased very little above control values of about 30 micra. For the seven remaining subjects, however, the Δt values changed very little as the subject maintained the unsupported hand in a horizontal position. For the remaining subject, the Δt values decreased very little from control levels of about 10,000 to 30,000 micra. The Δt values increased very little above control values of about 10,000 to 20,000 micra. For six of the nine subjects, the displacement amplitude and postural hand error also appeared to depend on the particular hand pathway(s) (Supported in part by USPHS Grant HD 14730.)

Two-deoxy-(14C) glucose as a marker for identifying acutely active skeletal muscle fibers in intact motor units (D. E. Teodoru,* T. A. Tran,* J. Herkovic,* and A. J. Berman. Department of Neurosurgery, V. A. Hospital, Bronx, N.Y. 10468) (spon. F. T. Hambrecht) Lab. of Neural Control, NINCDS, NIH and Lab. of Cerebral Metabolism, NIMH, Bethesda MD 20205.

The injection of trace amounts of (14C)-deoxyglucose (DG) permits autoradiographic evaluation of rates of glucose metabo­
limin in structures in the central nervous system (Sokoloff et al., J. Neurochem. 28:897, 1977). The uptake of DG has also been studied in rat muscle using this technique, where it was found that electrical stimulation greatly increases the uptake of tracer (Rapport et al. Exp. Neurol. 60:168, 1978). Our present aim has been to evaluate DG autoradiography as a label for muscle fibers belonging to an intact motor unit (i.e., those innervated by a single alpha motoneuron), which has previously been done by depleting intrafiber glycogen stores during prolonged stimulation (Edstrom & Kugelberg, C. Neuro., Neurosurg., & Psychiat. 31:424,1968). Glycogen depletion provides an independent "marker" for acutely active muscle fibers. In each of 5 adult cat flexor digitorum longus (FDL) and soleus (SOL) muscles (normal or self-reinnervated), a physiologically identified motor unit was stimulated repetitively while moni­
toring muscle unit force output (13 - 18 pulses in 40 Hz trains delivered once per second) to minimize the interinnervating motoneurons; see Burke et al., J. Physiol. 234:723, 1973). Immediately after the onset of stimulation, DG was injected intravenously (approx. 400 µg/kg, 0.1 ml saline) and simul­
ation was continued for 16 to 75 minutes. Between 20 and 72 minutes was allowed to elapse between DG injection and removal of the test muscle. Muscle bathing solution was then changed to a solution at -10°C and stored in a liquid nitrogen freezer. Freeze sections were cut at 10 µm in a cryostat at -20°C and air-dried. A cryostat was used to prevent "locking-in" to the crystallographic (100) planes of the collagen fibers, so that sections did not lie precisely parallel to the planes of the collagen fibers, so that sections did not lie precisely parallel to the surface of the muscle fibers. AAT and 2-DEOXY-(14C) GLUCOSE AS A MARKER FOR IDENTIFYING ACUTELY ACTIVE SKELETAL MUSCLE FIBERS IN INTACT MOTOR UNITS...
**EYE MOVEMENT CONTROL: THE EFFECTS OF JOINT SUPERIOR COLICUS AND FRONTAL EYE FIELD STIMULATION.** Sean True*, Peter H. Schiller*, and Janet Conway (Brown; R. Held) Dept. Psych., MGH, Cambridge, MA 02139.

Electrical stimulation of both the frontal eye fields (FEF) and the superior colliculus (SC) produces saccadic eye movements whose directions and amplitudes depend on the site of stimulation. Simultaneous stimulation at two loci within each of these structures produces a single saccade whose size and direction is a weighted function of the stimulus arising from each site (Robinson, D.A. Vision Res. 12: 1795, 1972. Robinson, D.A. and Fuchs, A.P. J. Neurophysiol. 32: 637, 1969). This averaging could be produced either within these structures or at a locus to which both project. The aim of this study was to determine what the interactions are between the FEF and the SC when they are jointly stimulated, either simultaneously or in succession. Stimulation of these structures was carried out in the alert monkey using implanted search coils to monitor eye movements. The animal's head was restrained during the experiment. Stimulation was between 10-600 μA, typically at 300Hz, 70-500 μsec train durations with 0.5 μsec pulse widths. For the most part microelectrodes were used for this purpose which permitted localization of each structure by recording prior to stimulation. Our results showed that paired stimulations produce results similar to those obtained when two sites are activated within each structure: for simultaneous stimulation the resultant saccade was the weighted average of the two saccades, where the relative intensity of stimulation is the weighting factor. With contralateral stimulation this can result in canceling of the specific response. When the two sites stimulated produce saccades in opposite directions. Sequential stimulation showed a refractory period in which the second stimulus has no apparent effect on eye movements. This refractory period corresponds to that seen with paired stimulation in a single structure. We did not see any case of interruption of a saccade by a following simultaneous stimulation in the cats.

These findings suggest that the FEF and the SC converge on a site in the brainstem where the averaging function observed is performed. Since we have previously shown that ablation of the SC does not alter stimulation elicited saccades from the FEF (Schiller, P.H. Brain Res. 122: 154, 1977.), it is unlikely that the effects reported here could be attributed to the FEF-SC pathway. (Supported by NSF grant #NS 86-8524 and NIH grants 5 R01 EY00676 and T31 T3F4784).

**THE UNUSUAL STRUCTURE OF TRAPEZIUS MOTONEURONS IN THE ADULT CAT.** J. S. Vanner* and P. K. Rose. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Anatomical studies of the spinal cord using Golgi stains have established that dendrites of spinal motoneurons have an organization dominated by rostrally and caudally directed dendrites usually 1000 μm in length. However, the consistency of this structural framework has not been systematically studied. We now report that the trapezius motoneurons have structural characteristics which are strikingly different from other spinal motoneurons. These neurons were identified in pairs in the cervical segments and are involved in head and forelimb movements.

Using chloral hydrate anesthetized cats, 23 trapezius motoneurons were antidromically identified and injected intracellulary with horseradish peroxide. Subsequent histological processing revealed that the trapezius motoneuron pool formed a column of neurons extending from the caudal C2 segment to the rostral C6 segment. The unusual feature of these motoneurons was their remarkably long rostrally and caudally directed dendrites. Occasionally these dendrites measured over 2000 μm from the soma and most exceeded 1000 μm in length. The total extent of the dendritic tree measured 3000 μm to 4000 μm. These rostral and caudal dendrites formed a distinct bundle which was unusually confined to the trapezius motoneuron nucleus. Ventromedial and dorsally projecting dendrites also measured over 1000 μm in length but lacked the distinct grouping of the rostrally and caudally directed dendrites. Although few in number, lateral dendrites projected into the lateral funiculus occasionally reaching the edge of the spinal cord.

The functional significance of these long dendrites will depend on their membrane characteristics. If the membrane resistivity of the trapezius motoneuron matches that reported for hindlimb motoneurons it is possible that synapses located on the distal dendrites will have little effect on the membrane potential of the soma. Alternatively, if the membrane resistivity is substantially larger, trapezius motoneurons may have electrotonic lengths similar to hindlimb motoneurons. (Supported by the Canadian Medical Research Council.)

**UNIT ACTIVITY IN THE MESENCEPHALIC RETICULAR FORMATION (MRF) ASSOCIATED WITH SACCADES AND POSITIONS OF FIXATION DURING A VISUAL ATTENTION TASK.**

David M. Weitzen and Bernard Cohen, Department of Neurology, Mount Sinai School of Medicine, New York 10029.

The MRF projects to the superior colliculus as well as to the ipsilateral and contralateral pontine reticular formation where rapid eye movements are generated. Electrical stimulation of the MRF causes contralateral saccades, and MRF lesions cause ipsilateral gaze palsy. We are studying whether MRF neurons participate in the generation of saccadic eye movements and positions of fixation. Unit activity is monitored while monkeys fix a small spot of light on a TV screen in the dark. When the spot dimms, they release a bar for a water reward. Eye movements are recorded with EOG. The spot is under computer control and can jump to new locations during the task. Animals sometimes break fixation to execute off-target saccades. Their eyes usually return to the spot with an on-target saccade after several hundred milliseconds.

**MOTOR SYSTEMS**

**UNIDIRECTIONALLY PROPAGATED ACTION POTENTIAL GENERATION IN PERIPHERAL NERVE BY SHORT STIMULI.** Christopher van den Honert*, and J. Thomas Mortimer. Dept. Biomed. Eng., Case Western Reserve University, Cleveland, Ohio 44106.

Electrical stimulation of peripheral nerve ordinarily elicits two action potentials propagated in opposite directions. A stimulation technique has been developed which generates a single, unidirectionally propagated impulse. Propagation in the opposite direction is inhibited by imposition of a longitudinal potential gradient in the extracellular medium which opposes the flow of excitatory action currents. The time course of that potential is roughly equivalent to the driving force behind the action currents, i.e. the action potential itself. Both stimulation and block are effected through an asymmetrical tripolar cuff electrode using a single central cathode. The total stimulating current is divided unequally between the anodes.

The method has been successfully demonstrated at rates up to 50 Hz in motor axons of cats using EMG measurements from medial gastrocnemius during sciatic nerve stimulation. The compound sciatic neurogram was recorded proximal to the electrodes. Stimuli consisted of regulated current pulses (1 - 10 μA, 0 - 8 ms) with a linear or exponential falling phase (0 - 10 ms). This technique may be used as an investigative tool, or to effect a clinical motor nerve block by introduction of antidromic impulses on the peripheral nerve. The block arises from the head on collision (and subsequent mutual annihilation) of the natural excitatory current and the artificially generated antidromic impulses. Such a collision block could provide control of spas ticity, particularly in the urinary sphincter muscle of spinal cord injury patients. Short term sphincter block would facilitate bladder voiding by electrical stimulation of the detrusor musculature.

This research was supported by NIH Grant No. NINEX N01 NS-2-2314 and GM 01090-16 Training Grant.

As part of a series of anatomical and physiological studies of effector mechanism controlling components of the feeding behavior of the pigeon, we have mapped the somatotopy of the jaw muscles in the Vth and VIIth cranial nerve nuclei using horseradish peroxidase histochemistry.

In the case of the protractor of the lower jaw, M. depressor mandibulae, the only jaw muscle innervated by the facial nerve, the labeled neurons were located totally and discretely within the medial facial nucleus lying in the center of the caudal pontine reticular formation. Labeled axons could be observed to leave the nucleus in dorsoventral directions before turning laterally, caudally and then laterally again to pass through the brainstem immediately ventral to the rostral portions of the lateral vestibular nucleus. The axons then pierced the descending trigeminal tract and exit the lateral brainstem caudal to their nucleus of origin.

In the case of the six jaw muscles within the orbit, all but the protractor of the upper jaw, M. protractor quadrati et pterygoidei, were represented within the largest or chief motor trigeminal nucleus located laterally within the medial facial nucleus. The adductor complex was represented ventrolaterally, M. pseudotemporalis superficialis ventrally, M. pseudo temporalis profundus dorsolaterally, and the two pterygoid muscles medially, centra1ly and rostrally. M. protractor quadrati et pterygoidei was represented in a more scattered group of neurons predominantly located between the medial facial nucleus and the caudal pole of the chief motor trigeminal nucleus, forming, as it were, a V-VII motor column.

Many labeled axons could be seen to exit the brainstem directly from the lateral and ventrolateral aspects of the otic and caudal pole of the chief motor trigeminal nucleus. However, many others ascend dorsally before turning laterally to join the discrete arching bundle of axons leaving the descending tract of the chief motor trigeminal nucleus, forming, as it were, a V-VII motor column.

The data suggest a somatotopic organization of the trigeminal motor complex but, just as there is considerable overlap in the position of the muscles with respect to each other within the orbit, so there is considerable overlap in their representation within the nucleus. In addition some facial and trigeminal sensory fibers innervating these muscles lie in close proximity. (Supported by NIMH Grant MH 08366 and a CUNY FRAP award to H.P.Z.)

MOTOR SYSTEMS

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MOTOR AND SOMATIC SENSORY CORTEX SINGLE UNIT BEHAVIOR WITH PASSIVE AND ACTIVE LIMB MOVEMENTS AND PORTION MAIN­

TENANCE IN PRIMATES. Jonathan R. Wolpaw. Neurology Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Four monkeys maintained hand position against a range of steady background forces. 50 ms force pulses, causing abrupt passive wrist flexion or extension, were superimposed. Pulse responses, background activity, and activity during active movement were recorded from 630 units in areas 4, 3, 1, 2, 5, and 7, which responded in 60 ms.

Three-quarters of task-related units in areas 4 and 2 behaved similarly with a pulse in one direction, background forces were recorded from 630 units in areas 4, 3, 1, 2, 5, and 7, which responded in 60 ms.

The particular correlation in areas 4 and 2 between unit behavior during passive movement, active movement, and position maintenance, combined with the shorter latency of area 2, is consistent with the possibility that short latency sensory input, largely from muscle stretch receptors, had significant control over area 4 unit behavior during active vs passive movements.


In the majority of normal subjects results indicate that the somatic stretch reflex in the gastrocnemius muscle is inhibited during the FSR of its antagonist muscle, the tibialis anterior. The inhibition occurred during all platform perturbations used (synchronous translateral, rotational and vertical motion in addition to translations). The velocity of slow components of both PRN and FSR with a constant rate of decay has durations that are determined by the initial velocity of the eye and therefore can never be described by a single time constant. The higher the eye velocity, the longer the duration of PRN; the lower the initial velocity, the shorter the duration and thus the apparent time constant. During sinusoidal rotations the effect of velocities and durations of eye movement are not of such magnitude to appreciably affect the mechanism underlying PRN.

In patients with multiple sclerosis a delayed inhibition of the Achilles tendon reflex and the functional stretch response in normal subjects and patients with multiple sclerosis. Marjorie H. Wollancott, Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

The EOG activity of four leg muscles [gastrocnemius (G), tibialis anterior (TA), hamstrings (H) and quadriceps (Q)] was monitored while freely standing normal and spastic humans were subjected to unexpected platform perturbations. The longer the duration of PRN, the lower the initial velocity, the shorter the duration and thus the apparent time constant. During sinusoidal rotations the effect of velocities and durations of eye movement are not of such magnitude to appreciably affect the mechanism underlying PRN.

In patients with multiple sclerosis a delayed inhibition of the Achilles tendon reflex in the G was observed concurrently with a delay in the tibialis anterior FSR.
Recent studies examining averaged EEG preceding voicing, the high frequency response of the EEG amplifier was seldom set beyond 50Hz. In the present study an electrode was placed on Cz and linked ear lobes served as reference. A ground was attached to the right forehead. The electrodes were connected to a differential amplifier with bandwidth 1Hz - 1000Hz and recorded on magnetic tape. A second channel of the tape was used to record a square wave trigger pulse obtained from the voice onset phonogram. Ten right handed female subjects were studied. Each subject produced 100 utterances of the words ATE and YES. A control consisted of random sampling of the EEG while the subject was still seated in a reclined chair, in a semi-darkened room with eyes fixed on a spot 30 inches distant and no voice production. Electromyograms from obicularis oris, obicularis oculi and masseter muscles were recorded on a third channel on tape. Analysis of the data involved backward averaging of at least 100 repetitions of the utterance using the voice onset to trigger the averaged sample periods of 128 msec were analyzed up to 668 msec preceding voicing. Averaged data were entered into a microprocessor for peak identification and latency assignment to all peaks and evoke going waves. A subsequent program then compared across individuals examining for identical peaks 21 msec over the range 0 - 668 msec. The results showed that in the time period, from 216 - 344 msec preceding speech, in at least 6 out of 9 subjects, there were 6 - YES peaks, 8 - ATE peaks and 2 - CONTROL peaks which were positive going and did not overlap. In this period 2 - YES peaks, 5 - ATE peaks and 3 - CONTROL peaks were negative going, with only one overlap of a control peak. No averaged electromyogram activity could be found in the time period 216-344 msec. These results suggest that distinct temporal patterns of activity exist preceding the production of an utterance and furthermore that these patterns are utterance specific across subjects. The precise role and origin of this averaged EEG activity is presently being investigated.


The location and inhibitory synaptic effects of medullary burst inhibitory neurones (BIN) on abducens (Abd) motoneurons has been described by Hikosaka and Kawakami (1978) in the decerebrate cat. The purpose of the present study was to quantitatively describe the activity of BINs in the alert cat and to determine their location, somadendritic morphology, axonal trajectory and termination sites. Neurons that exhibited a burst of activity prior to ipsilateral saccades were found in the medulla 0.7-1.0 mm from the midline at the level of the caudal Abd nucleus to 3.0 mm posterior to the Abd and 0.8-3.5 mm below the surface of the IVth ventricle. BINs discharged prior to and during ipsilateral voluntary saccades as well as the quick phase of vestibular and optokinetic nystagmus. The duration of the activity was related to saccade duration and the number of spikes was related to the saccade amplitude. BINs exhibited no activity during fixations but occasionally responded weakly during contralateral saccades. The axons of four BINs which demonstrated all the above characteristics were intracellularly injected with HRP in the alert cat. The soma of the injected neurons were located in the region described above and gave rise to 4-7 proximal dendrites. In one injected neuron, the dendritic tree extended over 0.6 mm rostrocaudally, 1.3 mm mediolaterally and 1.3 mm dorsoventrally. Each of the injected BIN axons terminated extensively throughout most of the contralateral Abd, although some areas were free of terminations. Its antigastrin by strychnine suggests a glycinenergic mechanism is involved in the expression of its effects in the ventral horn.

Supported by the Canadian MRC and the Quebec MRC.

A PHYSIOLOGICAL CLASSIFICATION OF MOTOR UNITS IN HUMAN FIRST DORSAL INTEROSSEUS (FDI) MUSCLES. Donald H. York, Tom W. Jensen, W. Casey Lenox* and John G. Rosenfeld*, Dept. of Physiological and Communications Disorder Unit, School of Medicine, University of Missouri, Columbia, MO 65212.

The question of whether there are identifiable event-related potentials preceding speech is quite controversial. The present study was designed to answer this question. In all previous studies examining averaged EEG preceding voicing, the high frequency response of the EEG amplifier was seldom set beyond 50Hz. In the present study an electrode was placed on Cz and linked ear lobes served as reference. A ground was attached to the right forehead. The electrodes were connected to a differential amplifier with bandwidth 1Hz - 1000Hz and recorded on magnetic tape. A second channel of the tape was used to record a square wave trigger pulse obtained from the voice onset phonogram. Ten right handed female subjects were studied. Each subject produced 100 utterances of the words ATE and YES. A control consisted of random sampling of the EEG while the subject was still seated in a reclined chair, in a semi-darkened room with eyes fixed on a spot 30 inches distant and no voice production. Electromyograms from obicularis oris, obicularis oculi and masseter muscles were recorded on a third channel on tape. Analysis of the data involved backward averaging of at least 100 repetitions of the utterance using the voice onset to trigger the averaged sample periods of 128 msec were analyzed up to 668 msec preceding voicing. Averaged data were entered into a microprocessor for peak identification and latency assignment to all peaks and evoke going waves. A subsequent program then compared across individuals examining for identical peaks 21 msec over the range 0 - 668 msec. The results showed that in the time period, from 216 - 344 msec preceding speech, in at least 6 out of 9 subjects, there were 6 - YES peaks, 8 - ATE peaks and 2 - CONTROL peaks which were positive going and did not overlap. In this period 2 - YES peaks, 5 - ATE peaks and 3 - CONTROL peaks were negative going, with only one overlap of a control peak. No averaged electromyogram activity could be found in the time period 216-344 msec. These results suggest that distinct temporal patterns of activity exist preceding the production of an utterance and furthermore that these patterns are utterance specific across subjects. The precise role and origin of this averaged EEG activity is presently being investigated.
TETANIC TENSION APPEARS TO BE A PERFECT PREDICTOR FOR RECRUITMENT OF PLANTARIS (PL) MOTOR UNITS IN THE CAT. Felix E. Zajac and Joel S. Faden. Univ. of Maryland, College Park, MD 20742.

Various studies on the whole population of motor units in a given muscle show that recruitment is statistically ordered either according to properties which reflect the "size" of the motoneuron or to properties which indicate the contractile strength of the motor unit. These findings are consistent with the fact that for the full range of motor units there are correlations between motoneuron size, conduction velocity (CV) of the motor axon and tetanic tension (TT) of the motor unit. However, such correlations between CV and TT are not high in mixed hindlimb muscles of the cat and are non-existent in the 61% subpopulation of fast-twitch (type F) motor units. This study (for methods see Neurosci. Abstr. 3:271, 1977) emphasized the recruitment order of type F units to distinguish whether the order is more related to axonal CV or to TT of the unit. Small intact filaments, each containing one PL axon, were dissected free from the L7 ventral root in decerebrate cats and axonal CV, TT and type (S, FR, F-int, FF) of each motor unit isolated was determined. Each filament was cut distally and discharges from its PL axon were recorded from its proximal end during monosynaptic reflexes. Discharge from one PL axon was compared with the presence or absence of discharge from another PL axon in another filament. Recruitment order of each pair of PL axons was compared with their motor unit properties. For each pair of units (both type S, or both type F, or of mixed type) the one with the smallest TT was recruited first (Table 1, col B). In contrast the unit with the slowest CV was recruited first in only 47% of type F pairs and in 73% of all pairs studied (Table 1, col C). These results are consistent with the probability that there is a monotonic trend between CV and TT (compare col C with D, Table 1). The probabilities were calculated on data from 134 PL motor units. TT is thus a better predictor for recruitment order in this motor pool than CV.

(supported by NIH grant NS 11518)

<table>
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<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
<td>Motor Unit Type</td>
<td>Smallest Tension Producing Unit</td>
<td>Slowest Conducting Motor Axon</td>
<td>Monotonic Trend Between CV and TT</td>
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<td>Each Pair</td>
<td>Recruited First</td>
<td>Recruited First</td>
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<td>100% (42/42)</td>
<td>73% (30/41)</td>
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NEUROCHEMISTRY
INHIBITION OF PYRIDOXAL KINASE BY GAMMA-AMINOBUTYRIC ACID.
Dept. of Chemistry, University of Maryland, College Park, MD. 20742.

Pyridoxal kinase is inhibited by gamma-aminobutyric acid (GABA), a neurotransmitter in the vertebrate central nervous system. Because GABA is synthesized by a pyridoxal-P requiring enzyme, this inhibition raised the possibility of a metabolic feedback loop for control of GABA synthesis. The results show that there was little inhibition of pyridoxal kinase by GABA at low concentrations of pyridoxal, but that the inhibition became stronger as the concentration of pyridoxal was raised. Similar results were obtained when 6-amino and 6-aminovaleric acid were substituted for GABA. Conventional models of enzyme inhibition did not fit the inhibition data and GABA did not inhibit when pyridoxamine was the substrate suggesting that GABA did not inhibit by interacting directly with the enzyme. Substrate depletion and direct inhibition of pyridoxal kinase by the pyridoxal-GABA imine were considered as alternative mechanisms. To distinguish between the two mechanisms, the equilibrium constants for pyridoxal-GABA imine formation were determined at pH 6.2, 7.3, and 8.0 (w=0.2 M, 37°C). The values were 0.23 M⁻¹, 1.4 M⁻¹, and 14.0 M⁻¹, respectively. The association constant for the pyridoxal-glycine imine was 1.0 M⁻¹ (pH 6.2). These values were used to calculate the concentrations of free pyridoxal and pyridoxal-GABA imine under assay conditions. The results showed that the pyridoxal concentration was not appreciably reduced by GABA indicating that inhibition was not the result of substrate depletion. This conclusion was supported by the failure of glycine to inhibit the enzyme. The calculated pyridoxal-GABA concentrations were used to analyze the inhibition data. The results showed that the pyridoxal-GABA imine behaved as a simple noncompetitive inhibitor with respect to pyridoxal thereby supporting the hypothesis that pyridoxal kinase is inhibited by the pyridoxal-GABA imine. In view of this indirect mechanism and the high concentration of GABA needed for inhibition, it seems unlikely that GABA inhibition of pyridoxal kinase represents a specific control point for GABA synthesis.

USE OF AN ANTICHOLINESTERASE AS PROBE IN THE STUDY OF MICROWAVE-INDUCED BLOOD BRAIN BARRIER CHANGES. Y. Ashani*, F. H. Henry* (Gambetti*, M.E. Velasco*, P. Gambetti, J.M. Sipple*). Division of Neuronephrology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106.

Mammalian neuregiments (DOP) have recently been shown to be made of three polypeptides: 200K, 145K and 68K daltons. These three polypeptides from NF-rich fractions of rat spinal cord (Shockey and Lasok, Trans Am Soc Neurochem 10, 1979) were separated in SDS slab gels; antisera to each of the NF polypeptides was raised in rabbits by injecting the corresponding bands from these gels. By indirect immunofluorescence and peroxidase-antiperoxidase (PAP) methods, antisera were seen to strongly their corresponding polypeptide and less intensely the other two NF polypeptides; in addition, a band migrating with tubulin was also stained. The 50K dalton polypeptide obtained from preparations of bovine brain filaments by the axonal floatation method (Lien et al, J Cell Biol 79:637, 1978) was not stained by either antisera. These results indicate cross-reactivity between the three NF polypeptides and antigenic identity between central and peripheral NF from different species.

Brain regions and activational state alter the influence of pH on tyrosine hydroxylase activity. Ann Acheson*, Linda Kennedy*, Gregory Kapust*, and Michael Zigmond. Departments of Biological Sciences and Psychology, and Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA. 15260.

Tissues from various rat brain regions were homogenized in Tris-HCl buffer, pH 6.0, and the high-speed supernatant was assayed for tyrosine hydroxylase (TH) activity in the presence of 75 µM L-(1-14C) tyrosine, 6MgM, catalase, and a dihydropteridine reductase regenerating system. We found that the influence of pH on the TH activity of these crude enzyme preparations varied with brain region. Striatum, a brain region rich in dopaminergic (DA)-containing nerve terminals, had a pH optima of 5.6. However hippocampus and cerebellum, regions rich in noradrenergic (NE)-containing terminals, had a pH optima in the range of 6.1 - 6.2. Brain areas with mixed DA and NE innervation had intermediate pH optima (frontal cortex, 5.8), or displayed biphasic pH curves with one peak between 5.7 and 5.9 and a second peak at 6.1 (olfactory bulb and hypothalamus). TH from areas containing cell bodies displayed less sensitivity to pH than TH from terminal regions. Activity from cell body areas was maximal over a broad range of pH values, which encompassed the individual pH optima of the terminal regions to which these cell bodies project (substantia nigra, 5.65 - 5.9; locus coeruleus, 5.95 - 6.3). We also found that activation of TH by phosphorylating conditions (0.2M cAMP, 1.0 mM MgCl₂, 0.5 mM ATP, and 2.0 mM theophylline) produced a decrease in the sensitivity of the enzyme to pH. Activated enzyme from striatum or hippocampus showed little decrease in maximal activity when assayed at a sub-optimal pH, thus increasing the effect of phosphorylation relative to basal activity. For example, the activity of TH increased under phosphorylating conditions was 4 times that of control activity at pH 6.5, whereas at pH 6.2, the activity was not significantly different from control.

These data support previous suggestions that TH exists in different forms in DA- and NE-rich brain regions. They also suggest the importance of terminal to control comparisons of TH activity and in studies of TH activation. (Supported in part by USPHS grants NS 29670 and 00058.

IMMUNOCHEMICAL STUDIES OF MAMMALIAN NEUROFILAMENTS. L. Autilio-Gambetti*, M.F. Velasco*, P. Gambetti, J.M. Sipple*. Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106.
The effects of several neutral amino acids on d-amphetamine and apomorphine-induced circling in rats with lesions in the substantia nigra. John M. Beaton and Issam H. Humaideh*, Neurology, Biochemistry and Neuropathology, Medical University of South Carolina, Charleston, S. C. 29403.

Neurological and spinal cord trauma result in degeneration of myelinated axons and causes paralysis in animals. Myelin proteins are progressively degraded, and ultrastructurally myelin is disrupted with vesicular exudation of myelin lipids and phagocytosis. The degradation of myelin proteins suggests a role for proteolytic enzymes and has prompted study of the nature and levels of proteases in intact and traumatized spinal cord. Levels of these enzymes in extracts from animals following cord injury have previously been reported (Trans. Am. 10:153). Since calcium accumulates in myelinolated axons in experimental trauma, some of these proteases may be activated by Ca++. Experimental spinal cord trauma was produced in rats by dropping 10 g from 30 cm upon exposed, dura-invested spinal cord. Extracts in distilled water, pH 5.6, were prepared from homogenates of normal and lesion regions of traumatized rats. The homogenate was centrifuged at 100,000 g (90 min.) and the supernatant freeze-dried and assayed for enzyme activity. The activity of neutral protease was assayed in 0.025 M phosphate buffer, pH 7.6, at 37°C (60 min.). One microgram of protein extract was incubated with 30 µg of purified myelin basic protein in a final volume of 0.08 ml. The incubation was carried out with and without CaCl2 (5 mM), EDTA (5 mM) and leupeptin (25 µg). The degradation of basic protein at pH 7.6 was greatly stimulated by Ca++ (5 mM) and a 69% loss of protein was observed. The addition of EDTA (5 mM) prevented the breakdown of basic protein. This Ca++-activated neutral protease is inhibited by leupeptin (25 µg) and 0.025 M CaCl2. These results suggest that like muscle there is a Ca++-activated neutral protease in spinal cord which is sensitive to leupeptin — and may play a significant role in the loss of myelin in spinal cord trauma. Thus the use of leupeptin may be useful in preventing the myelinolysis in spinal cord injury.

Supported by PHS Grant No. NSI1066.


The effect of different chromosomal constitutions on expression of a gene encoding a protein known to be present in the brain has been investigated. Cell fusions were performed using a mouse neuroblastoma clone, originally derived from the C1300 tumor, and a normal human fibroblast line, grown from a skin biopsy of a normal male neuroblastoma parent, NIE-1157TG2, is heteroploid and has no monoamine oxidase (MAO) or hypoxanthine phosphoribosyltransferase (HPRT) activity. This clone was derived from the line NIE-1157TG2 which was produced at a level of 100 pmol/min/mg protein. The fibroblast parent, GM316, has a normal diploid male complement of 46 chromosomes, and has both HPRT and MAO activity. Monoamine oxidase activity in this line is expressed at a level of 16 pmol/min/mg protein. Following hybridization, parental cells were selected against by growth in the presence of hypoxanthine, aminopterin, thymidine (HAT) and ouabain. Hybrid cells were cloned in HAT medium to select for retention of the human X chromosome which contains the gene coding for HPRT. Ten hybrid clones retained the human X and all mouse chromosomes, but only a few human autosomes. These clones expressed varying levels of MAO activity from 0 to 100 pmol/min/mg protein. This finding suggests that if there is only one gene determining expression of this enzyme, it is probably not on the human X chromosome. Hybrid clones with high MAO activity were subcloned under non-selective conditions allowing random loss of the human X chromosome. Several subclones had MAO activities of >1000 pmol/min/mg protein, over 100-fold higher than either parent. The MAO-activity in these hybrid clones could result from gene amplification or overexpression on the human gene(s) and/or retention of the human gene(s) coding for this enzyme. The overexpression of activity reflects the interaction of a few specific human chromosomes with a predominantly mouse neuroblastoma genotypic. Studies are underway to identify which human chromosomes are retained and whether any alterations in the mouse chromosomal complement occur in subclones with high MAO activity. Electrophoretic techniques are being used to distinguish the species of origin of the MAO.

1331 THE EFFECTS OF SEVERAL NEUTRAL AMINO ACIDS ON D-AMPHETAMINE AND APOMORPHINE-INDUCED CIRCLING IN RATS WITH LESIONS IN THE SUBSTANTI A NIGRA. John M. Beaton and Issam H. Humaideh*, Neurosciences Program, University of Alabama in Birmingham, B'ham, AL 35294.

Following unilateral destruction of the nigrostriatal dopamine system little behavioral change may be noticed. However, compounds which either directly stimulate post-synaptic dopamine receptors (e.g. apomorphine) or increase synaptic dopamine content (e.g. amphetamine) produce unilateral turning. The directionality of the turning is dependent upon whether the test compound acts pre- or post-synaptically. Amphetamine acts pre-synaptically and produces turning in the direction ipsilateral to the lesion. Apomorphine which acts post-synaptically induces contralateral turning. There is a large body of evidence which indicates that neurotransmitter receptors (e.g. apomorphine) or increase synaptic dopamine content (e.g. amphetamine) produce unilateral turning. The directionality of the turning is dependent upon whether the test compound acts pre- or post-synaptically. Amphetamine acts pre-synaptically and produces turning in the direction ipsilateral to the lesion. Apomorphine which acts post-synaptically induces contralateral turning. There is a large body of evidence which indicates that the brain levels and synthesis of the putative neurotransmitters, serotonin, dopamine and norepinephrine, can be modified by acute or chronic alterations of the peripheral levels of the large neutral amino acids (e.g. leucine, valine). The present study was carried out to study the effects of pretreatment with L-valine, D-valine and L-leucine (250 mg/kg) on the response of substantia nigra lesions, treated with various doses of d-amphetamine (2.4 mg/kg) or apomorphine (0.25, 0.5 or 1.0 mg/kg). The rats were lesioned electrolytically in the right substantia nigra. At least 10 days were allowed between the surgery and drug testing. The various amino acids tested were all injected subcutaneously in the back of the animal. Unilateral lesions of either amphetamine or apomorphine. After injection of the drug the animals were placed individually in the center of a circular open field and turning behavior was recorded for 20 min. at 0, 15, 30, 45 and 60 minutes after the administration of the drug. L-valine pretreatment at 250 mg/kg resulted in a marked decrease in amphetamine-induced circling in rats with unilateral substantia nigra lesions but had no effect upon apomorphine-induced circling. It is suggested that this suppression of the amphetamine effect is due to the lowering of brain tyrosine levels by the administration of the L-valine. L-leucine induced similar effects. D-valine at a similar dosage was inactive in suppressing either the amphetamine or the apomorphine-induced circling. This suggests that tyrosine is not surprising since the carrier system for the entry of these amino acids into brain is thought to be stereospecific.

This work was supported in part by Intramural Faculty Research grant R2-6602.
Cyclic GMP pm/mg protein
0.39 ± 0.04 (5)*
0.53 ± 0.03 (4)*
0.38 ± 0.05 (8)*
0.33 ± 0.01 (4)*
0.53 ± 0.03 (4)*
0.37 ± 0.03 (4)*
0.30 ± 0.02 (5)*


Acetylcholine and cyclic GMP each have excitatory effects on α receptor association kinetics are observed for iodinated toxin electroplax nicotinic receptors, and peculiarities of toxin-radiolabeled toxins from sites on Torpedo californica kinetides of toxin-receptor interaction may reflect the nature of the chemical modification used to introduce radiolabel, rather than the intrinsic complexity of native toxin-membrane bound receptor interactions.

Supported by the Division of Biomedical and Environmental Research of the U.S. Department of Energy under contract No. W-705-ENG-48.


The rat hippocampus receives a cholinergic innervation which is derived from cell bodies located in the medial septal nucleus, through the diagonal band, and plays a role in the intermediate-lateral regions of the septum (Brain, 90:521; Brain Res., 119:1). Considerable evidence has been produced to support the view that cyclic GMP is involved in muscarinic cholinergic neurotransmission.

Whole hippocampi from decapitated adult Sprague-Dawley rats were pre-incubated for 30 min at 37°C in Eagle's Medium containing 5 mM theophylline, and then incubated in the same solution containing 2.2 mM CaCl2 and bethanechol. Samples were analyzed for cyclic GMP and protein (J. Neurochem., 32:1033).

**INCUBATION CONDITIONS** CYCLIC GMP pm/mg protein ± S.E.M. (No. samples)

0.25 Min., 500 µM Bethanechol 0.33 ± 0.01 (4)*
0.50 Min., 500 µM Bethanechol 0.30 ± 0.02 (5)*
1.0 Min., 500 µM Bethanechol 0.34 ± 0.02 (8)*
2.5 Min., 500 µM Bethanechol 0.39 ± 0.04 (5)*
3.0 Min., 500 µM Bethanechol 0.42 ± 0.03 (4)*
5.0 Min., 500 µM Bethanechol 0.53 ± 0.03 (4)*
Controls 0.091 ± 0.02 (7)

**0.25 Min., 100 µM Bethanechol 0.21 ± 0.01 (6)*
0.50 Min., 250 µM Bethanechol 0.37 ± 0.03 (4)*
2.5 Min., 100 µM Bethanechol 0.33 ± 0.03 (4)*
2.5 Min., 1000 µM B ethanechol 0.46 ± 0.07 (6)*

*p<0.001 (Student's t-test).

The cyclic GMP increases were calcium-dependent and were blocked by atropine sulfate. Other studies suggest that the same may give rise to cyclic guanosine monophosphate production in the basal and apical dendrites of hippocampal pyramidal cells. Acetylcholine and cyclic GMP each have excitatory effects on rat hippocampal pyramidal cell isolated populations to electrophysiological stimulation of the pathway from the medial septal region to hippocampal field CA1 (Fed. Proc., 37:524). It thus seems reasonable to infer that cyclic GMP is generated by activity in the muscarinic cholinergic projection from the septum to the hippocampus (Supported by NS 11650 to THU).

Treatment of rats with a single dose of 3-acetyl pyridine (3AP, 75 mg per kg, i.p., 12h) results in severe neurological impairment in surviving animals. Histological studies have shown that 3AP produces partial degeneration of facial, hypoglossal and ambiguous nuclei and complete destruction of the inferior olive nucleus with resulting degeneration of cerebellar climbing fibres. These lesions were detectable as early as 7 hr after a single injection of 3AP and 48 hr after injection, few, if any intact climbing fibres remained (Desclosin and Escubi, Brain Res., 77, 349 (1974)).

Rats showed decreased locomotor activity, catalepsy as well as a distinctive muscle rigidity 6 hr after the administration of 3AP. Anomalities of gait (ataxia) were apparent 13 hr post-treatment and by 24 hr all rats showed loss of righting reflex.

Measurement of amino acids in several discrete regions of brains of affected animals 72 hr after 3AP treatment revealed the following:
(a) A decreased tyramine concentration in cerebellum and medulla oblongata.
(b) Decreased glutamic acid concentration in cerebellum, medulla oblongata, cerebral cortex, striatum, hippocampus and olfactory bulbs.
(c) No changes in concentrations of GABA, glycine or aspartic acid in any region of brain studied.

These findings are consistent with the hypothesis that glutamic acid may be the excitatory neurotransmitter of cerebellar climbing fibres. The importance of the amino acid changes in the development of catalepsy and rigidity produced by 3AP will be discussed.

(Supported by l'Association Canadienne de l'Ataxie de Friedreich, F.B.R. and E.H. were supported by the Medical Research Council of Canada.)

1378 ISOLATION AND CHARACTERIZATION OF A PROTEOLIPID ASSOCIATED WITH (3H) SPIROPERIDOL BINDING ACTIVITY. Yvonne C. Coates, John H. Lee and Richard M. Caughton*. Maria S. C. Costa* and Vanda O. Breakefield — Dept. of Human Genetics, Yale Univ. Sch. Med., New Haven, CT 06510

Three methods of polyacrylamide gel electrophoresis were used to analyze the structure of [3H]-labelled monomeric monoamine oxidase (MAO) from cells with A and B activity. These two forms of the enzyme are known to differ in their substrate selectivity, drug sensitivity, and tissue distribution. It has not been clear whether these differences result from intrinsic variation in the structure of MAO or from extrinsic modulation by its membrane microenvironment. The irreversible inhibitor, 3H-pargyline, was bound to crude mitochondrial fractions from rat hepatoma line H17, with A and B activity, and rat glioma line C6, with A activity. Specific labelling of only the A or B forms of hepatoma cells was controlled by pre-incubation with selective A and B inhibitors. Electrophoresis in sodium dodecyl sulfate (SDS)–polyacrylamide gels revealed a single protein band of MW 57,000 for both forms. Electrophoresis in non-equilibrium pH gradient gels again showed a single labelled band for both forms, indicating they also have a similar charge; however, binding of 3H-pargyline to the A form was more labile than to the B form under these conditions. To further examine the structure of the A and B forms of MAO, the labelled protein bands obtained by SDS–polyacrylamide gel electrophoresis were subjected to proteolysis and electrophoresis in SDS–polyacrylamide gels to identify peptide fragments. Site-specific proteases can be used against fully solubilized and denatured MAO to identify discrete differences in amino acid composition or modification, independent of associated lipids. Analysis of fragments of MW >10,000 generated by Staphylococcus aureus V8 protease revealed unique peptide fragments present only when the B form of the enzyme was labelled. The other three fragments observed were common to both A and B forms.

In conclusion, the catalytic poly peptides responsible for A and B types of activity are similar in MW and charge, but differ in their characteristics of [3H]-pargyline binding and in their covalent molecular structure.


One of the principal aims of the present study was to determine whether the A and B forms of MAO were structurally different. To this end, we have carried out a detailed analysis of the structure of the A and B forms of MAO. These studies have been supported by the National Institutes of Health (Grant HD-09878) and by the American Cancer Society (Grant #00-16).

In conclusion, we have shown that the A and B forms of MAO are structurally different and that these differences are due to the presence of a single amino acid residue difference. The A form contains an arginine at position 36, while the B form has a lysine at the same position. This difference is responsible for the difference in substrate specificity of the two forms. The A form is specific for MAO, while the B form is specific for DOPA. The difference in substrate specificity is due to the presence of a single amino acid residue difference. This difference is responsible for the difference in substrate specificity of the two forms.

(Supported by National Institutes of Health Grant HD-09878 and American Cancer Society Grant #00-16.)

The lipid and elemental composition of ten control and eleven multiple sclerosis (M.S.) brain samples were determined using sensitive analytical techniques. Semi-quantitative lipid analyses were done using thin-layer chromatography. No gross abnormalities in the lipid composition of normally appearing M.S. white matter were observed. However, in white matter containing plaques, a dramatic rise in free fatty acids was noted in eight M.S. brains. Sulfatides appeared to be decreased in plaque-enriched white matter, and cholesterol esters were usually elevated.

Ten elements, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, and Zn, were within the analytical range of detection, and accurate values for these were obtained. Twenty other elements were below detectable limits. White matter from controls was compared with normally appearing white matter from M.S. brains, and marked differences were noted in some elements, as listed below:

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</table>

The significance of these elemental alterations is not known. The possibility that demyelination could be related to the differences is supported by data obtained on a M.S. plaque, whose Ca, Fe, P, and Zn levels were: 51.1, 205, 3071, and 15.3 μg/g respectively. There appeared to be no substantial elemental differences between white and grey matter.
Guclidean Protein Metabolism in the Monocularly Deprived Split-Brain Pigeon. Dale Deutsch and Anton Reiner, Depts. of Biochem and Psych. SUNY Stony Brook, Stony Brook, New York 11790.

Transplantation of the dorsal supraoptic decussation disrupts interhemispheric transfer of monoculturally learned visual discrimination tasks in birds. In the present study, brain glucose and protein metabolism were studied in such "split-brain" pigeons after monocular visual deprivation. Split-brain pigeons were prepared (R.E. Meier, Psychol. Rev., 80, 220, 1973) by decapitation with a razor, followed by intracardial perfusion with 10% neutral buffered formalin. The brain was removed, placed in 10% formalin, and stored for one week. The brain was then cut on a horizontal plane, and the hemispheres were dissected from the brain. Two slices of the telencephalon were prepared and incubated in a Krebs-Ringer bicarbonate buffer containing 10 mM glucose, 10 mM ascorbic acid, and 0.05 mM dopamine-β-hydroxylase (DBH) for 3 hours at 37°C. Membrane and soluble fractions were prepared and fractionated on one- and, in some cases, two-dimensional electrophoresis. Comassie blue staining and fluorography were employed for detection of protein levels and radioactive label, respectively, and the resulting patterns were analyzed on a scanning microdensitometer. No significant differences were detected in the protein patterns from the deprived and control telencephalic hemispheres or between the two optic lobes.

These results suggest that functional alterations in brain activity, as evidenced by the 2-DG technique, may not necessarily be accompanied by changes in protein levels or synthesis. However, it is possible that protein changes occur within the telencestria or visual Wulst, for example, and that these changes be accompanied by changes in protein levels or synthesis. How­ever, it is possible that protein changes occur within the telencestria or visual Wulst, for example, and that these changes were masked by analyzing the entire telencephalic hemisphere. We thank Harvey J. Karten and Melvin V. Simpson for their help and assistance during these experiments.

Glucose and Protein Metabolism in the Monocularly Deprived Split-Brain Pigeon. Dale Deutsch and Anton Reiner, Depts. of Biochem and Psych. SUNY Stony Brook, Stony Brook, New York 11790.

The study of protein and glucose metabolism in the brain of the split-brain pigeon after monocular visual deprivation has revealed some interesting findings. The brain glucose metabolism was unchanged, while the protein metabolism showed significant changes. The protein metabolism was studied using 2-deoxyglucose (2-DG) and autoradiography. The 2-DG technique was employed to detect changes in protein synthesis and degradation. The results showed a decrease in protein synthesis in the telencephalic hemispheres of the deprived side compared to the control side.

The study also revealed that the protein metabolism was affected in specific regions of the brain. The telencephalic hemispheres and optic lobes showed a decrease in protein synthesis, while the thalamus and hypothalamus showed an increase in protein synthesis. The changes in protein metabolism were correlated with changes in glucose metabolism, as determined by the 2-DG technique.

The results of this study suggest that the functional changes in the brain caused by monocular visual deprivation are accompanied by changes in protein metabolism. These changes may be related to the development of new neural connections and the reorganization of existing neural networks. The study also highlights the importance of studying the metabolic changes in the brain in response to environmental changes, as these changes may have long-term effects on brain function.

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The precise role of the Type I cells and the affrent nerve terminals in the sensory transduction process of the carotid body has not been experimentally clarified. Ultrastructural studies have repeatedly demonstrated abundant dense-cored vesicles in the Type I cells, characteristic of catecholamine storage. The studies have also identified synaptic-like contacts between the Type I cells and the affrent nerve terminals. Recent biochemical studies have characterized the synthesis and release of dopamine from the carotid body, the most abundant catecholamine in this organ. Pharmacologically, it appears that exogenous dopamine is excitatory in the rabbit and inhibitory in the cat with respect to its effect on afferent fiber discharge. In order to gain further insight concerning the site of action of dopamine, we have attempted in this study to detect and characterize dopaminergic receptors in the rabbit carotid body.

Pairs of rabbit carotid bodies were rapidly removed from pentobarbital-anesthetized animals, cleaned of surrounding connective tissue, and incubated in a water-bath-shaker at 37°C for 20 min. In a modified Tyrode's solution containing different concentrations of ³H-spiroperidol, with and without 0.02 µM (+)-butaclamol. Following incubation the tissue was washed for 6 min. in ice cold Ringer's solution. The weight of each carotid body was determined to the nearest tenth of a microgram. Levels of tissue radioactivity were obtained using liquid scintillation spectrometry. Specific binding was defined as the amount of spiroperidol bound in the presence of (+)-butaclamol less the amount bound in the presence of (+)-butaclamol. The data were analyzed on a Scatchard plot. The Kd of binding was calculated to be 0.38 nM, and 4.17 pmol of receptors was found per gram of tissue. These values compare favorably with those obtained in the rabbit carotid body, and in the rabbit mesencephalon for similar concentrations of dopamine receptor agonists and antagonists. These results suggest that the protein and glycoprotein composition of SPN from ethanol lungs are abnormal. The observed SPN abnormalities may result in altered synaptic connectivity and function.

This work was supported by a grant from the National Council on Alcoholism. Dr. Mary Druse-Manteuffel is the recipient of a Schewepp Foundation Career Development Award.
1353 THE EFFECTS OF ORALLY ADMINISTERED U-0521 (COMP), AN INHIBITOR OF CATECHOL-O-METHYLTANSFERASE. Stanley Fahn and A.L.N. Prasad* Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032 U-0521 given intraperitoneally to rats was an effective inhibitor of COMT, but was toxic in high dosages when administered with levodopa. In this present study, U-0521 was administered p.o. to rats. To effectively inhibit methylation of orally-administered levodopa, U-0521 had to be administered 30-60 min prior to levodopa. U-0521 blocked methylation peripherally and in brain for up to 4 hrs following levodopa administration. A dose-response curve revealed that 400 mg/kg of U-0521 produced maximum inhibition of methylation of levodopa. Lowering the dose of levodopa did not increase the percent of inhibition of methylation, indicating that the active sites of COMT were not saturated at a dose of 250 mg/kg or 400 mg/kg of U-0521. Of potential therapeutic importance was the observation that doses of up to 500 mg/kg of U-0521 were not toxic to rats.

1354 CYCLIC NUCLEOTIDES IN THE CONE-DOMINANT RETINA OF HIBERNATING GROUND SQUIRREL. Debora B. Farber, David Chase*, Dennis Souza* and Richard N. Lolley, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, and V.A. Medical Center, Sepulveda, CA 91434. The retina of the 13-line ground squirrel contains almost exclusively cone photoreceptors. Upon entering or emerging from hibernation, cone photoreceptors of the ground squirrel undergo major changes in morphology, some of which have been reported by Beme and Young (Invest. Ophthal. 15, 451, 1977) and by Kubara (Invest. Ophthal. 14, 457, 1975). In addition to a reduction in the length of cone outer segments, synaptic ribbons are decreased in size and number during the early stages of hibernation and are re-formed upon emergence from hibernation. Changes in synaptic ribbon morphology are specific to visual cells because synaptic ribbons of the bipolar terminals are unchanged during hibernation. Cyclic AMP levels of non-hibernating, dark-adapted ground squirrels exceed those of cyclic GMP by about 8-fold; white light causes a significant reduction in cAMP levels exclusively. During hibernation at 6°C, cAMP levels are modified but cGMP content is not affected. During the first days of hibernation, retina cAMP levels rise above the values measured in non-hibernating squirrels in a dark-room environment. Following one and up to three months of hibernation, cAMP levels are stabilised at a value which is higher than those in awake animals in the light. Within three days after arousal from hibernation, cAMP levels return to pre-hibernation levels, and cone morphology re-forms simultaneously. These observations support the hypothesis that hibernation alters cyclic nucleotide metabolism and cAMP metabolism. The present studies also support the hypothesis that cone photoreceptors contain higher levels of cAMP than cGMP. (Supported by NIH Grant EY05651, Research to Prevent Blindness, Inc., and the Medical Research Service of the Veterans Administration.)

1355 SYNTHESIS AND RELEASE OF CATECHOLAMINES BY THE CAT CAROTID BODY: EFFECTS OF HYPOXIC STIMULATION. S. Fidone, C. Gonzalez* and K. Yoshizaki* Dept. Physiol., Univ. Utah Col. Med., SLC, UT 84108. The presence of large amounts of catecholamines (CA), mainly dopamine (DA), in cat carotid body is well established. The inhibitory effect of DA on the chemosensory activity in this animal is apparently different from its action in the rabbit, rat and perhaps dog, where its effects are reportedly mainly excitatory. In previous studies, we have characterized in detail the process of biosynthesis and release of DA from the rabbit carotid body. Because of the difference in DA actions in rabbit vs. cat, it was of interest to compare certain aspects of DA metabolism in these two species. In experiments dealing with the synthesis of CA by cat carotid body, hypoxic stimulation was affected by exposure of the animals for a 3 hr. period in an atmosphere of 10% O2 in N2 immediately prior to removal of the carotid bodies for incubation in modified Tyrode's solution containing 3H-tyrosine or 3H-dopa. In the release experiments, the carotid bodies were first pre-loaded with labelled DA synthesized from 3H-tyrosine, and then mounted in a superfusion system that permitted simultaneous collection of the superfusates and monitoring of the chemosensory discharge from the carotid sinus nerve. Hypoxic stimulation involved superfusion with media equilibrated with different O2 tensions.

Our findings can be summarised as follows: 1) The kinetic characteristics of the process of synthesis of CA in the cat carotid body are different from those in the rabbit. However, and this is comparable to that described for other catecholaminergic structures. 2) As in the rabbit carotid body, hypoxic stimulation in the cat does not modify the rate of synthesis when 3H-dopa is used as precursor but with 3H-tyrosine the synthesis is increased by more than 80% above control value. 3) There is spontaneous release of 3H-Da from cat carotid body as well as a stimulus-related increase in 3H-Da release which is proportional to the intensity of the hypoxic stimulation. The increase in chemoreceptor discharge from the carotid sinus nerve parallels the increase in 3H-Da release. These findings are similar to our previous observations with rabbit carotid body. Thus, although other studies in our laboratory have shown clear differences between rabbit and cat with regard to the short-term effects of hypoxia on tyrosine hydroxylase activity in the cat carotid body, the present study fails to uncover any striking differences in the short-term synthesis of DA or its pattern of release in these two animals. Supported by USPHS grants NS07938 and NS12636.
DETERMINATION OF N,N-DIMETHYLTRYPTAMINE IN HUMAN CEREBROSPINAL FLUID (CSF), INTRAVENTRICULAR FLUID (IVF) AND RAT BRAIN USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY. Joachim P. Gerdemann, John M. Beaton, George B. Brown and John R. Smythies, Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

The hallucinogen N,N-dimethyltryptamine (DMT), which may be produced by aberrant N-methylation of tryptamine, has been postulated as a psychoactive responsible for the symptoms of schizophrenia. The studies to be presented here were carried out to determine (1) if exogenously administered DMT is taken up into the synaptic regions of rat brain and (2) if DMT is an endogenous substance in human CSF or IVF. Adult male Long-Evans rats were injected IP with 5, 10 or 20 mg/kg α, β, β-tetraduterated DMT (D4-DMT) 30 min. prior to sacrifice. Purified synaptic vesicles were then prepared from the brains of 4 groups of three rats. The vesicles were extracted with methylene chloride and the extract derivatized with heptfluorobutyryl imidazole (HFB). Samples of the derivatized extract were then analyzed on a GC/MS. The electron impact mass spectrum of the DMT as HFB - derivative exhibits m/e 58.1 as the base peak due to the alpha-cleavage of the tertiary amino group. All peaks of 1 to 3 u were chromatographed over a four-foot glass column, 2mm i.d., of 2% SP-2250 at 150°C for 2 min, followed by a programmed temperature increase of 10°C/min. to 280°C. Under the conditions of injection, DMT eluted at 2.2 min. Identification of the two compounds was based upon retention time and selected ion monitoring of the mass fragments 58.1 and 129.0 for DMT and 60.1 and 129.0 for D4-DMT. The exogenously administered D4-DMT was found to be present in the rat brain synaptosomes after intraperitoneal injection of 5, 10 or 20 mg/kg. Quantitative analyses of human CSF and IVF were achieved by using D4-DMT as an internal standard. The occurrence of endogenous DMT could be shown in some human samples. The data below indicate approximately 100 ng DMT per 1 ml, occurring in a sample of human IVF.


In previous studies we established that with varying levels of hypoxia the release of DA from rabbit carotid body increased with increased chemosensory discharge. In the experiments described here, we investigated the effects of removal of Ca++ from the superfusion medium and its substitution by Mg++. Carotid bodies were pre-loaded with 3H-DA synthesized from 3H-tyrosine and then mounted in a superfusion system that allowed collection of the superfusates together with the simultaneous recording of the sensory discharges from the carotid sinus nerve. The stimulus period consisted of 5 min. of superfusion with 20% O2 in N2-equilibrated media. During the remaining periods, the carotid body was superfused with 100% O2-equilibrated media. In the first and last cycles, superfusions were performed with media of normal ionic constitution, while in the middle two cycles the media contained either 0 Ca++ or 0 Ca++ plus 2.1 mM Mg++. We found that during superfusion with normal-ionic media, the stimulus-related release of 3H-DA was 10-13 times greater than basal release levels and the chemoreceptor discharges were 6-9 times basal levels. Neither the basal discharge nor the basal release of 3H-DA were affected by the 0 Ca++, high Mg++ media, but the stimulus-related release of 3H-DA was increased only 1.3-1.5 times control values and the sensory discharges were increased only 2-6.4 times control levels. These data suggest that the hypoxia-induced release of DA from rabbit carotid body is Ca++-dependent, and that DA release may be important for the full expression of chemosensitivity in this organ.

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BRAIN ALKALINE PHOSPHATASE IN MAMMALIAN SPECIES. David J. Goldstein and Harry Harris*. Dept. Human Genetics, School Medicine, University of Pennsylvania, Philadelphia, Pa. 19104

At least three gene loci are involved in the expression of the various forms of human alkaline phosphatase (ALP); one coding for the placental form of the enzyme; at least one coding for the intestinal form; and at least one coding for the liver, bone and kidney forms. These three classes of human alkaline phosphatase can be discriminated one from another by their behavior with certain inhibitors, by thermostability, by electrophoresis and by immunologic characteristics.

We studied the alkaline phosphatase in brains and livers from several species including rodents (mouse, rat, guinea pig, hamster), a carnivore (cat), ruminants (cow, sheep) and man. By thermostability and degree of inhibition with L-phenylalanine, L-homogarginine and L-phenylalanylglucyclglycine, the brain ALP in all these species, including man, closely resemble the liver/bone/kidney ALP.

When comparing the activities of brain ALP of certain adult and of fetal animals, a large developmental difference was discovered. Fetal guinea pig brain contained nearly twenty-fold greater activity than an adult. Newborn lamb brain contained about five-fold greater activity than adult in the cortex and cerebellum and about a ten-fold greater difference in the mid and hind brain. In addition, fetal rat brain contains six-fold greater activity than adult rat brain.

These findings raise interesting questions about the role of alkaline phosphatase in the brain.


Enkephalins, a membrane bound dipeptidyl-carboxypeptidase, can degrade enkephalin in a highly specific fashion. Its high affinity for enkephalin, regional variation paralleling the opiate receptor and changes during addiction suggest that the enzyme is responsible for the inactivation of the neurotransmitter (Malfroy et al., Nature 276:523, 1978; Swerts et al., Eur. J. Pharmacol. 53: 209, 1979). We have developed a binding assay using a 3H-peptide ligand displaying uniquely high affinity for the enzyme. Peptide specificity for the binding site is identical to that of enkephaline activity assayed by measuring enkephalin degradation. Binding and catalytic activity of the enzyme in mouse and rat brain have been explored in the soluble and purified state. Alterations in binding and catalytic activity have been evaluated as a function of changed sensitivity to opiates, angiotensin and Bradykinin also substrates for enkephalinas. (Supported by USPHS grant DA-00266)

We have immunohistochemical techniques it has been shown that the olfactory marker protein (OMP) is localized in the mature olfactory neurons of adult mice. The immature stem elements of the neurons and the supporting cells do not stain with the peroxidase-antiperoxidase method, and are presumed not to contain the protein (G. A. Monti Graziadei et al., J. Histochem. Cytochem. 25, 1977). The peroxidase-antiperoxidase method consequently be used to determine the proportion of mature versus immature elements in embryos as well as in animals of different age. Differentiated olfactory neurons, with an apical dendrite and an axon, are present at ten days gestation in the deepest part of the olfactory pit. At 14 days the olfactory epithelium contains a continuous layer of differentiated elements. The OMP begins to appear at this time, when it is present in few scattered neurons. The number of neurons containing the OMP rapidly increases. At 17 days of gestation positive neurons are found all through the sensory area, however some regions of the neuroepithelium are devoid of marker. In neonatal mice the common histological stains evidence an epithelium which is not substantially different from the one of the adult animals. However, the mature neurons stained by antiserum to OMP are restricted to a small band which occupies the greater portion of the olfactory pit. The number of stained neurons increases till 30 days post-natally, when an equilibrium is reached between mature and immature elements. However, the proportion of immature neural elements is not constant all through the sensory sheath. Even in the adult animals there are zones where the immaturity is prevalent of the mature ones. These data are in agreement with previous morphological and autoradiographic observations (Graziadei and Monti Graziadei, J. Neurocytol. 8, 1979) which have shown in the neuroepithelium zones where the neurogenic process is more/less vigorous. These zones have been termed accordingly: active and quiescent zones.

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1362 MOUSE PNS MYELIN: AN ANALYSIS OF PROTEIN COMPOSITION FOR YOUNG AND ADULT QUAKING AND NORMAL MICE. S. Greenfield*, H. J. Weiss*, H. Sarvage, S. W. Brostoff*, and E. L. Weihl (Departments of Neurology, Biochemistry and Immunology, Medical University of South Carolina, Charleston, S. C. 29403).

In order to determine whether the abnormalities in ultrastructure and lipid content of Quaking (Qk) mouse PNS are accompanied by alterations in the protein composition of PNS myelin, mouse sciatic nerve myelin was isolated from 21-25 day old and from adult normal (N) and Qk mice. The yield of myelin isolated from adult Qk mouse sciatic nerve was two-thirds of control. In contrast to the initial characterization of the protein compositions of Qk and N adult mouse PNS myelin (Greenfield et al., Trans. Am. Soc. Neurochem. 55, 1978), a recent report (Matthieu, Biochem. J. 173, 399, 1979) has shown a drastic decrease in basic protein content of Qk compared to N sciatic nerve myelin. In our current studies, as before, we find that most differences are limited to 30% or less. Exceptions to this are 1) a 42% decrease in P0 (the small basic protein analogous to Bg of CNS myelin - Milak et al., Trans. Int. Soc. Neurochem., 1979) of Qk vs. N at 21-25 days, and 2) a two-fold increase in P1 by inosine and guanosine (IC50 0.40 and 0.312, 1978) in Qk vs. N adults. Analysis of myelin protein preparations in a basic protein RIA shows only 20-30% less basic protein for Qk vs. N mouse.

Our analysis of myelin from young and adult mice also suggests the occurrence of an age-related shift towards an increase in the ratio of the small to large PNS basic proteins (P0 and P1, respectively) similar to that observed for Bg and B1 of CNS myelin. This shift during maturation may not be as pronounced in Qk as in N mice. The P0 and P1 proteins have been shown by SDS gel and RIA analysis to be analogous to Bg and B1 (Milak et al.), the pathology in the Qk mouse appears to have a lesser effect on the protein composition of PNS compared to CNS myelin.

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Adenosine and adenine nucleotides depress the spontaneous firing of rat cerebral cortical neurons (Cas. J. Physiol. Pharmacol. 52, 1226, 1974). Inosine, adenine and 2'-deoxyadenosine have a similar but weaker action. Hypoxanthine and guanosine are virtually devoid of depressant activity. The benzodiazepines, diazepam and flurazepam, also depress the firing of cerebral cortical neurons. However, the depressant actions, at least in part, of diazepam may be due to an increased uptake of adenosine into brain cells. The diazepam binding site may therefore be the adenosine uptake site.

We have previously shown that adenosine is taken up into astrocytes in primary cultures with a Km of 3.4 µM and a Vmax of 0.36 n mol/min per mg protein (J. Neurochem. 31, 1978). In the present work the effects of diazepam, 2'-deoxyadenosine, inosine, hypoxanthine and guanosine on adenosine uptake by cultured astrocytes were studied at an adenosine concentration of 5 µM. Uptake of adenosine was greatly inhibited by diazepam (IC50 20 µM) and 2'-deoxyadenosine (IC50 < 0.25 µM), less efficiently by inosine and guanosine (IC50s of 5 and 100 µM respectively) and only little, if at all, by hypoxanthine (IC50 72.5 µM). These findings are consistent with the hypothesis that diazepam may elicit its depressant actions by increasing the levels of extracellularly released adenosine and further suggest that compounds such as inosine and 2'-deoxyadenosine exert their depressant actions at least in part, by a similar mechanism, namely by inhibiting the uptake of adenosine into brain cells. The failure of iontophoretically applied weak agonists or negative substances such as inosine, adenine and 2'-deoxyadenosine, which like adenosine displace diazepam from its binding site in brain tissue (Life Sci. 24, 851, 1979), to antagonize adenosine induced depression is a further indication that the diazepam binding site in brain tissue cannot be identical with the adenosine receptor. The diazepam binding site may therefore be the adenosine uptake site.

Supported by the Medical Research Council.

1364 EXPERIMENTAL ALLERGIC NEURITIS IN THE RAT - STRAIN DIFFERENCES IN THE RESPONSE TO BOVINE P2 PROTEIN. P. M. Hoffman*, J. M. Powers* and S. W. Brostoff* (SPON: G. F. Young). V.A. Medical Center, Charleston, S.C. and Departments of Neurology and Pathology (Neuropathology), Medical University of South Carolina, Charleston, S.C. 29403.

Bovine peripheral nerve myelin antigens were tested for their ability to produce experimental allergic neuritis (EAN) in three inbred and one outbred strains of rat. Each antigen was emulsified with complete Freund's adjuvant (CFA) (Difco 1377A) and injected intradermally into both hind footpads in a total volume of 0.2ml/animal. Clinical disease and histologic lesions characteristic of EAN were prominent in Lewis rats but not in Sprague-Dawley, Buffalo or Wistar rats, when bovine P2 or whole PNS myelin was used. Lewis rats responded with the most severe lesions and clinical signs when challenged with bovine PNS myelin (2 mg/animal) which contains P2 protein in its native configuration. Isolated P2 protein (200 µg/animal) produced definite but less severe disease. Reduction of disulfide bonds by treatment with mercaptoethanol enhanced the neurotoxicity of P2 protein. When rats were challenged with bovine galactocerebroside in CFA in the absence of bovine P2, no clinical or histologic evidence of EAN was present in any of the strains tested. These studies confirm the marked susceptibility of Lewis rats to EAN induction with isolated, intact bovine P2 protein (Nature 277, 140, 1979) in contrast to rabbit, guinea pig and mouse, which show partial or no susceptibility. As we earlier noted in the rabbit (Nature 246, 752, 1977), the conformation of the P2 protein may be important in determining its ability to induce EAN.

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PHOSPHORYLATION OF RIBOSOME-ASSOCIATED PROTEINS IN CEREBRAL CORTEX

whether TH is in the (+P) or (-P) form (Trans. Am. Soc. Neurochem., 10, 1979). It was found that TH activity in the LC was increased by only 10% (378±56 to 432±58) while no increase was observed in adrenal. The increase of NaF on TH activity in crude tissue preparations (39,000g supernatant) was 30%: in SN (1108±194 to 1435±256) and CN (2718±302 to 3543±350). In noradrenergic neurons, the prior addition of NaF to the assay mixture of dopaminergic neurons significantly in the brain. Current investigations in our laboratory indicate that phosphorylation of the proteins involved in these phenomena. The present studies were designed to examine whether the effect of NaF is tissue-specific, and in CN from 1886±292 to 3446±540. In noradrenergic neurons, the effect of NaF on TH activity in crude tissue preparations appeared to be increased in phenylalanine-treated animals, but the specific factors responsible for this increase have not yet been identified. These results suggest that variations in the phosphorylation of translational factors may be involved in alterations in brain protein synthesis in experimental hyperphenylalaninemia. [Supported by a Canadian MRC Fellowship and research grants NS-13295 from the National Institutes of Health and NS-259 from the United Cerebral Palsy Research and Educational Foundation.]

EFFECTS OF SODIUM FLUORIDE, A PHOSPHATASE INHIBITOR, ON TYROSINE HYDROXALATE ACTIVITY IN VATX.

We have proposed that native tyrosine hydroxylase (TH) from the caudate nucleus of rat brain is composed of a mixture of active and inactive forms, and that phosphorylation of the enzyme by cAMP-dependent protein kinase and ATP converts the inactive (-P) to an active (+P) form (PNAS, 75:4744, 1978). This implies that an active form (+P) can be converted to an inactive form (-P) by protein dephosphorylation which may be mediated by an endogenous protein phosphatase in crude tissue extracts. If so, addition of NaF, a commonly used phosphatase inhibitor, to the TH assay mixture may block dephosphorylation, thereby increasing TH activity. In the present studies we have examined the effect of NaF on TH activity in crude tissue preparations (39,000g supernatant) in which protein phosphatase may be present.

In order to examine whether protein kinase (+P) and tissue-specific, various tissues containing TH were used, including substantia nigra (SN) and dopaminergic neurons, locus ceruleus (noradrenergic neurons) (LC) and adrenal medulla. Addition of NaF to the assay mixture of dopaminergic neurons significantly (P<0.001) increased TH activity upon using the in vivo to 4.3 (0.13 to 1.3) in SN. In addition, NaF increased both TH activity and TH mRNA.

ADENOSINE REGULATES CYCLIC AMP FORMATION IN SPINAL CORD THROUGH ALPHA-ADRENERGIC RECEPTORS. David J. Jones and Laurie F. McKenna.

Our studies from our laboratory have demonstrated norepinephrine-stimulated formation of cyclic AMP in rat spinal cord tissue slices to be mediated by both alpha and beta-adrenergic receptors. The regulation of the alpha component of this response by adenosine was investigated in the present studies using incubated rat spinal cord tissue slices. 150-175 mg/45s/. Sprague Dawley rats were decapitated and cervical and sacral spinal cord removed. The cord was then sliced bi-directionally using a McIlwain tissue chopper set at 300 µm and slices pre-incubated for 40 min in oxygenated Krebs-Ringer bicarbonate buffer. Aliquots of tissue were subsequently subjected to incubation flask with fresh buffer for an additional 15 min, at which point NE, phenylephrine (PE) or isoproterenol (ISO) were added. Antagonists phenoxycyanazone (PBA) or propranolol (PPL) and/or adenosine were added 10 min prior to NE, PE or ISO addition. Following addition of agonists the incubations were carried out for 10 additional min at the end of which tissue slices were homogenized and cyclic AMP measured by radioimmunoassay. All units are pmol cyclic AMP/mg protein.

The addition of 10-5 M NE stimulated cyclic AMP accumulation from 7.3±0.7 to 41.0±3.2. The prior addition of 10-5 M adenosine potentiated the response with NE to 60.5±5.3 (p<0.01 vs NE). Adenosine alone produced an insignificant increase in cyclic AMP accumulation (10.5±1.8). The alpha-adrenergic receptor stimulation caused only a slight increase in cyclic AMP (10.9±0.9, NS). However, when added in presence of adenosine, a 4-fold increase occurred (45.4±6.3). ISO at 10-5 M stimulated cyclic AMP formation (7.3±0.5, p<0.025) which was not altered in the presence of 10-5 M adenosine. 10-5 M PBA blocked completely the increase in cyclic AMP due to adenosine with either NE or PE, whereas PPL did not. In addition the adenosine receptor blocking agent phenolamine (10-5 M) also blocked the enhanced response due to adenosine with either NE or PE. It is evident from this data that adenosine modulates the alpha receptor response for cyclic AMP accumulation in spinal cord tissue slices.

Supported by NIH grant NS15456
SYNAPTOSOMAL DOPA DECARBOXYLASE (DDC) AND TYROSINE HYDROXYLASE (TH) ACTIVITIES ARE SENSITIVE TO CHANGES IN AMINO ACID TRANSPORT. Ira R. Katz* (SPON: Nansie S. Sharpless). Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Previous studies of the extent to which amino acid transport limited catecholamine formation have compared the rates of transport into heterogeneous synaptosome preparations with synthetic rates. For greater specificity, I studied the effects on DDC and TH activity of various amino acids in synaptosomes incubated with the substrate carrier, using rat striatal P2 fractions. DCC and dopamine formation from tyrosine (requiring both TH and DDC) were assayed by release of 14CO2 from carboxyl-labelled DOPA and tyrosine-3H, by release of 3H from 3,5-DH-tyrosine. In intact synaptosomes, DCC is inhibited by amino acids of the "L" transport system. Lys, ornithine, or by detergrent, gives variable effects on DCC activity; the remaining activity is consistently insensitive to amino acid inhibition. The requirement for synaptosome integrity implies interaction at the level of transport. At 2μM DOPA, various amino acids at 100μM gave inhibition as follows: leucine, 50%; methionine, 32%; valine, 11%; phenylalanine, 60%; l-chlorophenylalanine 50%; and aminoacyclohexanecarboxylic acid, 50%. Leucine was chosen for further study. With 2μM DOPA, 12%, 38%, & 50% inhibitions were seen at 10, 25, & 50μM Leucine. Though inhibition was not strictly competitive, it decreased with increasing DOPA. In studies of the interaction between leucine and 3-hydroxybenzylhydrazine, (NSD-1015), a potent DCC inhibitor, it was found that partial inhibition by NSD-1015 led to potentiation of leucine inhibition. This is consistent generally. Leucine inhibition by stimulating DOPA efflux was not predicted for inhibition via inhibition of influx. The above is corroborated by studies of amino acid transport in bulk synaptosomes: at 2μM DOPA, 100μM leucine, Efflux of DOPA is stimulated by leucine. Efflux of leucine is also enhanced by amino acids in the medium is expected. Inhibition of TH is also observed. At 2μM Leucine, 25, 100, and 200μM Leucine at 0.5μM tyrosine. 14CLeuc is inhibited by 48% at 100μM Leucine and 64% at 250μM while 3H release is inhibited by 33% at 100μM Leucine. The remaining activity is consistent with stimulated efflux of DOPA from the synaptosomes. Thus, efflux of substrate and intermediate amino acids must be considered a potential regulatory steps in dopamine biosynthesis.

Supported by NIA Grant number AG 01478.

NEUROCHEMISTRY

PERSISTENT ALTERATION IN BRAIN AMINO ACIDS FOLLOWING HYPOXIA AND LESIONS. A. S. Kimes and M. K. Shellenberger. Behav. Res. Res. Center,  Kansas Univ. Med. Center, Kansas City, KS 66103. We have previously reported persistent changes in the levels of catecholamines when adult rats were exposed to carbon monoxide (CO) for 4-6 weeks (Kimes et al., J. Pharmacol. Exp. Ther., 212: 466, 1979). As part of an on-going study we have also examined the effects of CO-induced hypoxia and cortical lesions on the levels of various amino acids (Asp, Ser, Glu, GABA) suspected of being involved with neurotransmission.

Four groups of animals were used. The first group contained male and female adult rats (4 months old) which were exposed to CO (0.42% in air flow 4.5 l/min) until comatose and to a point near respiratory failure. In a second group of adult rats, a discrete area of cortex was removed bilaterally by aspiration under light pentobarbital-anesthesia taking care to leave the olfactory tracts and sagittal sinus intact. The last two groups were controls: one was of rats exposed to air in the chamber and a second control group consisted of sham operated animals. The brains of animals from these groups (3/group ≥ 6) were removed 4-6 weeks after the treatment and rapidly frozen. These brains were sectioned and the PCA soluble fraction analyzed for amino acid levels. The following sections were used: frontal cortex, striatum, midbrain-hypothalamus and pons-medulla. The amino acids were separated by ion exchange chromatography and quantitated fluorometrically.

CO exposure resulted in a significant elevation of Asp (+80%) and Gln (+100%) in the striatum (p< .05) in both sexes. Significant increases were seen in striatal Asp and Gln were found in the brain operated animals suggesting that these experiments enhanced synaptosomes. LE and CO exposure led to a significant decrease of Gln in the striatum while the Asp response was not consistent across sexes. Male animals showed an increase (+5%) in Asp in hypothalamus and sural nerve but not in the midbrain. Females showed a significant change (+10%) in striatal Asp with no alterations elsewhere. In the cerebellum, four amino acids, Asp, Ser, Gln, and GABA were revealed. Only Asp showed an extent large enough to be reflected in changes of levels.

In summary, exposure to CO-hypoxia and frontal cortical lesions results in persistent increases in amino acids in the levels of catecholamines. It remains to be determined whether these findings reflect altered metabolic or neurotransmitter functions.

Supported by USPHS Grant GM 27739. ASK was supported by DRIED Research Service Award HD 07666 from NICHD.

NEUROTRANSPORT FROM BUNGARUS FASCICULUS VENOM. T.P.A. Kruck* and D.M. Lane⁵ (SPON: J.D. Vickers). Dept. Biology, York University, Toronto, Canada, M3J 1P3. The increasing cost and limited availability of Bungarus multicinctus venom has encouraged us to examine the venom of the related snake Bungarus fasciolatus as a source of specific neurotoxins and in particular a sub-type for α-Bungarotoxin. Crude venom was fractionated on several different column materials with optimal separation being obtained with CM30 (Bio Rad) which is consistent with stimulated efflux of DOPA from the synaptosomes. Thus, efflux of substrate and intermediate amino acids must be considered potential regulatory steps in dopamine biosynthesis.

Supported by NIA Grant number AG 01478.

DEPLETION OF BRAIN TAURINE CONTENT BY GUANIDINOETHYL SULFONATE. Hugh E. Laird, Shirley Lippincott* and Ryan J. Huxtable* Dept. of Pharmacology, University of Arizona, Tucson, Az 85721. Further advances in our understanding of taurine in the brain await the development of pharmacological antagonists to taurine, and the finding of methods to modify taurine concentrations in the brain. We have found that the administration of guanidinoethyl sulfonate (GES) leads to a drop in taurine concentrations in all regions of the brain. For example, after 9 days of guanidinoethyl sulfonate therapy the concentration of taurine in the cerebellum, inferior colliculi, hypothalamus, midbrain, and cerebral hemispheres were reduced by 30, 30, 37, 51, 43 and 44%, respectively. After 20 days of guanidinoethyl sulfonate treatment taurine was depleted by 49, 59, and 60% in the cerebral cortex. On the other hand, females showed a significant decrease (p< .05) in striatal Asp with no alterations elsewhere. In the cerebellum, four amino acids, Asp, Ser, Gln, and GABA were identified. Only Asp showed an extent large enough to be reflected in changes of levels.

In summary, exposure to CO-hypoxia and frontal cortical lesions results in persistent increases in amino acids in the levels of catecholamines. It remains to be determined whether these findings reflect altered metabolic or neurotransmitter functions.

Supported by USPHS Grant GM 27739. ASK was supported by DRIED Research Service Award HD 07666 from NICHD.
1373 CYCLIC GMP AND CYCLIC AMP INCREASES IN SPECIFIC BRAIN REGIONS FOLLOWING CENTRAL CHOLINERGIC STIMULATION. Robert H. Lenox, G. Jean Kant, and James L. Meyerhoff. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington, DC and Dept. Psychiatry, University of Vermont, Burlington, VT.

Cyclic nucleotides are known to be involved in the central cholinergic system. Increases in cyclic nucleotides are known to be involved in the central cholinergic system. Increases in cyclic nucleotides have been demonstrated in a number of organ systems including the brain. Cholinergic agonists have shown to increase the levels of cyclic AMP in the cerebellum of both rats and mice. Changes in reported levels of cyclic AMP have either been variable or nonexistent. We have proceeded to examine more closely the regional effects of choline chloride on cyclic nucleotides. Following central cholinergic stimulation in the brain of the rat.

Male albino rats, 60 gm, weighing between 250-300 grams were anesthetized with ketamine and decapitated. The heads were dissected and the brains were removed. The brain was placed on a dissecting tray and cut into 18 sections using a bovine brain atlas. Each section was homogenized and assayed for cyclic nucleotides. The tissue from each section was assayed for cyclic GMP and cyclic AMP.

The levels of cyclic GMP in the cerebellum of animals receiving oxotremorine increased significantly (0.71 ± 0.10 to 1.00 ± 0.20 nmoles/mg tissue) as old levels in several other regions. i.e., brain stem, midbrain, and hypothalamus, increased. The levels of cyclic AMP in all regions increased, with the most dramatic increase in the midbrain and thalamus. Increases were not seen in the hypothalamus and interpeduncular region, with the most dramatic increase in the midbrain (1.15 ± 0.13 to 14.63 ± 2.74 nmoles/mg tissue). There were no significant differences in cyclic AMP levels between the groups. However, significantly increased cyclic GMP levels were seen in all regions examined.

The regional pattern of increase in cyclic AMP levels in the midbrain and thalamus of HMD embryos is similar to the response following central cholinergic stimulation (Meyerhoff et al., Life Sci., 1979) consistent with the behavioral observation of tremor. The increased levels of cyclic AMP, particularly in the midbrain and the thalamus, are in agreement with the behavioral observation of tremor. Following central cholinergic stimulation in the brain of the rat.

Supported by Grant A-4914, from NRC to Canada of D.L.L. and Grant 5759 from the Medical Research Council of Canada and Grant 15-14 from the Ontario Heart Foundation to M.P.R.  

1374 PARALLEL EFFECTS OF PHYSOSTIGMINE ON CHOLINERGIC ENZYMES IN BRAIN AND IN BLOOD. S.-C. Liang* and L. L. Esu* (Spon. V. Davis) Brain and Developmental Neurology Research Center, Texas Research Institute of Mental Sciences, 1300 Moursund Ave., Houston, Texas 77030.

Physostigmine, an anti-ChE drug known to reverse the central effects of atropine in man, has been extensively used to treat Parkinsonian conditions and extrapyramidal side effects resulting from long term therapy with phenoxythiazine and butyrophenone. Furthermore, it has been observed that cholinergic antidepressants have a tautodynamic dyskinesia and in the related condition of Lottun's Chorea. Recently, it has been described that I.V. and oral administration of physostigmine has led to an improved condition in manics and in chronic schizophrhenics. There have been no reports however, over the correlation between changes in cholinergic enzyme activity in brain and in blood. We have investigated each possible correlation in mice after i.p. administration of physostigmine. For this study, ten adult male BALB/c mice were divided into two groups: five received 30 ul of physostigmine (0.3 mg/kg, i.p.) and 5 received 30 ul of distilled water (i.p.) injections. Thirty min. following the administration, mice were sacrificed by decapitation. Selected brain regions (corpus striatum, hippocampus and remainder of brain) were immediately dissected and homogenized in 0.05M a-phosphate buffer (pH 7.5) for determination of cholinergic enzyme activity. Physostigmine, 500 ul of 1 M stock solution, was added to each homogenate and incubated at 37°C for 60 min. Cholinesterase activity was determined in the presence and absence of 10 M physostigmine. The basal cholinesterase activities (ACHE; CAT and AcCoA hydrolase) of whole brain from each mouse was collected into 100 ul of ice cold 0.15 M saline. Fractionation of the homogenates was performed and the RBC AChE plasma ChE, and AcCoA hydrolase activities were measured. Our results indicate that in the physostigmine treated mice (n=5) AcCoA hydrolase activity was increased in corpus striatum (125%) and the RBC AChE activity was slightly but not significantly decreased (125%) and AcCoA hydrolase was not changed in whole brain. (b.) ACHE was significantly decreased in corpus striatum but not changed in hippocampus or remainder of brain. CAT activity was significantly decreased in any of the brain regions examined. In conclusion, we have observed a parallel reduction in plasma ChE in specific brain regions, corpus striatum and CAT in corpus striatum. These observations may provide a basis for study of the possible cholinergic mechanism in schizophrenia.


The physiologic distribution of specific binding sites for [3H]kainic acid was determined in 14 species of invertebrates and vertebrates. The highest level of binding was observed in the brain of the frog (Xenopus laevis), followed by the spiny dogfish (Heterorhodus francisci), the goldfish (Carassius auratus) and the chicken (Gallus domesticus). Significant specific binding was noted in some (e.g. hydra littoralis), but not all of the phylogenetically lowest forms tested. In most cases, specific binding was to both high and low affinity sites. The exceptions were the cockroach brain (Periplaneta americana), which had high affinity sites, and the crayfish brain (Procambarus clarkii), which had high affinity sites in the chaper. In the spiny dogfish, the smooth dogfish and the chicken, the highest level of binding occurred in the corpus striatum; less occurred in the forebrain and the least in the medulla; in the mammalian species, the highest level of binding occurred in the brain structures; less in the cerebellum and least in the medulla.  

Bacterial plaques of the saturation isotherm for [3H]kainic acid revealed similar kinetics of binding for frog whole brain, rat forebrain and human parietal cortex with two apparent populations of binding sites: Kd=50-300 nM and Kd=14 nM. While binding in the frog and dogfish cerebella and brain caudate nucleus occurred exclusively at high affinity sites, binding was to both low affinity sites in the cerebellum of chicken, rat and man. In the three species studied, [3H]kainic acid and unlabeled kainic acid was the most potent inhibitor of [3H]kainic acid specific binding. L-Glutamic acid was 20- to 200-fold less potent than kainic acid and 2,500-fold less potent than its L-isomer. Reduction of the isopropyl amide chain of kainic acid to form dihydrokainic acid decreased the affinity of the compound 100-fold. The Hill coefficient derived from these inhibition studies was 1.0 for unlabeled kainic acid, it was approximately 0.5 for L- and D- Dihydrokainic acid. These results are compatible with negative cooperativity. These studies demonstrated a widespread distribution throughout the animal kingdom of specific binding sites for kainic acid in neuronal tissues. The characteristics of the receptor sites were remarkably similar from primitive vertebrates to man, suggestive of an endogenous neuronal system that has not been appreciated in mammals.
NEUROCHEMISTRY


Taurine has been considered as a putative neurotransmitter in the Central Nervous System; evidence for this function is not yet conclusive and an alternative role as a modulator of nerve excitability has also been suggested (Mandel and Pasantes-Morales, Rev. Neurosci. Vol 3 pp. 157, 1978).

Binding to synaptic membranes has been a useful tool in the identification of post synaptic receptors for neurotransmitter candidates. It has been considered that the interactions of neurotransmitters with post synaptic receptors is a sodium independent process which becomes more apparent in frozen and thawed membranes, while the opposite is true for other kinds of receptors - uptake sites for instance.

In the present work, we studied the characteristics of [3H]-taurine binding to membranes from the rat retina, from the rat cerebral cortex and dorsal root ganglia. Parallel studies were carried out for GABA, a recognized central inhibitory neurotransmitter. Membrane preparation and binding assay were carried out following the general procedure of Ems and Snyder (Brain Res., 100: 81, 1975).

Taurine binding to chick retinal membranes in the absence of sodium was extremely low (0.014 ± 0.004 p moles/mg protein); under the same conditions, GABA binding was of 0.49 ± 0.16 p moles/mg protein. In the presence of sodium, maximum taurine binding was 22.9 p moles/mg protein. In rat cerebral cortex membranes, taurine binding was extremely low in the presence of sodium and practically undetectable in the absence of sodium. GABA binding, although considerable in the presence or absence of sodium, was higher in the later condition. In membranes obtained from dorsal root ganglia, in which no synaptic type taurine binding was observed, [3H]-taurine or [3H]-GABA was observed to be frozen and thawed membranes in the absence of sodium. In freshly prepared membranes in the presence of sodium, taurine binding could not be detected whereas GABA binding was consistently observed.

According to the present results, an interaction with the characteristics ascribed to post synaptic receptor binding was not observed for taurine in any of the preparations studied.


Proteins from goldfish optic tectum were separated by high resolution two-dimensional polyacrylamide gel electrophoresis. The separation pattern obtained from gels stained with coomassie blue yielded two hundred polypeptide spots. Individual spots were quantitated by computer-automated integration of the optical density of films of the gels. This was accomplished by approximating the optical density surface representing the scanned film with a set of best-fit two-dimensional Gaussians. This method resulted in volume integrals accurate to ±5% in six minutes of processing time on a DEC 1099 computer.

Multiple gels of tectal homogenates were compared by a local measurement algorithm which uses the Gaussian parameters for the protein spots in each gel as an input. This method of comparison singled out 5 micrograms of creatine kinase added to a comparison sample of tectal homogenate as the only difference between two otherwise identical samples. The application of this method to two-dimensional gels of 350- or 140-labeled proteins from goldfish tectum, toad optic nerve, or human fibroblasts allowed the quantitation of relative differences between gels containing over five hundred spots. This technique is currently being employed to identify goldfish tectal membrane proteins which are altered in concentration during regeneration of the optic nerve.

1380 COMPARATIVE EFFECTS OF PENTOBARBITAL, KETAMINE, EPHEDRINE AND HALOTHANE ON CEREBROSPINAL FLUID DYNAMICS IN THE RAT. J. D. Mann, S. T. Cookson*, E.S. Mann*, Departments of Neurology and Anesthesiology, University of North Carolina 27516.

Four different anesthetic agents were studied to determine their effects on mechanisms regulating intracranial fluid dynamics in adult albino Sprague-Dawley rats. Comparable anesthetic dosages were achieved by anesthetic-induced hypothermia, halothane 40 mg/kg/hr i.v.; enflurane, 2.5% in oxygen; and pentobarbital 40 mg/kg i.p.; ketamine hydrochloride 40 mg/kg i.v.; enflurane, 2.5% in oxygen; and halothane 1.0% in oxygen. Following induction of anesthesia with one of these agents, animals were paralyzed with pancuronium bromide (0.1 mg/kg i.v.), artificially ventilated at a respiratory rate of 30 to 35 breaths/min, 37°C, and heart rate, arterial blood pressure and blood gases were monitored throughout the experiment. Artificial CSF buffered to pH 7.35 was then infused at a rate of 0.2 ml/hr or 1 ml/hr, and peritoneal, arterial or lumbar CSF was monitored for pressure responses. Infusions were performed at multiple rates (between 5 and 30 ul/min) until steady state pressure was achieved for each infusion rate. A previously described mathematical model of CSF dynamics was used to assess intracranial compliance, CSF outflow resistance at the arachnoid villi, and CSF formation rate (J.D. Mann, Ann. Neurol. 3:156-165, 1978).

Blood pressures were comparable in all groups (102 ± 14 SD mm Hg) with the exception of the enflurane animals (77 ± 13 mm Hg). Resting CSF pressure was lowest for the pentobarbital anesthetized rats (25 ± 19 mm H2O) and highest for the enflurane group (130 ± 48 mm H2O). Significant suppression of the hypothermic effect of pentobarbital with either halothane or ketamine (3.9 ± 1.24 4 mm and 3.29 ± 1.87 ul/min). However, CSF formation was enhanced 55% (p<.05) with halothane, and 80% (p<.01) in animals anesthetized with enflurane. Both anesthetics suggest a complex interaction between anesthetic agents and the various mechanisms which regulate intracranial fluid dynamics, including CSF formation, pressure responses of the meninges, and arachnoid villi. The biochemical bases for these changes remain to be determined. This work was supported by NIH grant R29NS21804.
FRAAGMENTATION OF SUBSTANCE P BY BRAIN AND PITUITARY CATHEPSIN B. Neville Marks and Alojz Suhar*. Center for Neurochemistry, Rockland Research Institute, Wards Island, N.Y. 10035
Neurosecretory regions of rat brain have been found to contain high levels of cathepsin B relative to other areas suggesting a possible involvement in the processing of neuropeptides. In other tissues cathepsin B has been implicated in the activation of inactive precursors to yield active hormonal peptides. In addition, cathepsin B is considered to play a key role in the intracellular turnover of proteins by non-lysosomal proteolysis in the CNS. Enzyme, therefore, was purified from human pituitary and bovine brain over 40 fold using classical procedures yielding in some preparations an enzyme with a single protein band. Both tissues contained a family of related SAMA hydrolases hydrolyzing benzoyl-arg-argyl-β-naphthylamide of which only one resembled the classical cathepsin B purified from other tissues (pH optima 6.5 in presence of -SH groups. Inhibition by low concentrations of Ac-Leu-Leu-arginyll or leuphin).

Purified enzyme from both sources cleaved a number of CNS components including histones, neurophymins, myelin basic protein, β-lipotropin and β-endorphin. The enzyme was selective since it did not cleave albumin, γ-globulin or casein, and showed low or no activity with hemoglobin and polylysine. Surprisingly, among the best substrates tested was the unlabeled peptide substance P (Arg-Pro-Lys-Pro-Gln-Gln-Ph-e-Gly-Glu-Leu-Met-NH₂).

Since cleavage products of substance P show a spectrum of activities dependent on the test system utilised then proteolytic enzymes may play a role in regulation. Among the products isolated after incubation of substance P with purified brain and pituitary cathepsin B was the tetrapeptide Phe-Glu-Glu-Leu indicating probable cleavage at the Glu-Phe and Leu-Met bonds. Cleavage at the first bond would generate a C-terminal pentapeptide that would retain ability to contract guinea pig gut in vitro whereas it should lose the property of depolarisation of frog spinal motorneurons. Loss of Met,NH₂ from the protease or substance P would lead to Inactivation. Cathespin B of brain and pituitary would be added to the list of enzymes known to degrade substance P and which include aminopeptidases, neutral endopeptidases, and a specific prolyl endopeptidase. Supported In part by grant NS-12578

A RAPID, SENSITIVE METHOD FOR THE SEPARATION AND MEASUREMENT OF TRICYCLIC ANTIDEPRESSANTS AND THEIR PSYCHOACTIVE METABOLITES IN HUMAN SERUM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. D. M. Martin*, A. L. C. Potts*, and M. S. Gold. Yale University School of Medicine, New Haven, CT 06520, Psychiatric Institutes of America and Psychiatric Diagnostic Laboratories of America, Summit, NJ 07901
Recent studies indicate that the measurement of serum levels of tricyclic antidepressants (TCA) and their psychoactive metabolites may facilitate the effective pharmacological management of endogenous depression. Existing High Performance Liquid Chromatographic (HPLC) techniques employing both normal and reverse phase technologies are limited by column deterioration or complex mobile phase formulation respectively, making their routine application in the clinical laboratory difficult.

Tricyclics were extracted from one (1.0) ml of patient serum into a basic organic phase, freeze dried and taken to dryness under a stream of nitrogen. The residue was injected into an isocratic system of 0.025% butylamine in methanol flowing at 1.4 ml/min. through a column of fully porous silica. Detection was in the ultraviolet at 254 nm, Amtriptyline (A), Imipramine (I), Nortriptyline (N) and Desipramine (D) respectively were separated in a single injection (Fig). Unknowns were quantitated by comparison of peak height ratios to standard curves generated against spiked blanks run in parallel. Over five hundred patient samples have been run using this technique yielding a mean day-to-day coefficient of variation of less than 7%. As little as 5 ng of each drug can be reliably detected in one (1.0) ml serum. Recovery of spiked blanks ranged from 65-75% increasing with concentration as reported in the literature. The precision, reproducibility, ease of sample preparation and simplicity of instrumentation make this technique exceptionally well suited for therapeutic monitoring of tricyclic antidepressants.

AURATUS L.) D.F. Matheson*, R. Oei* and Betty I. Roots, Dept. Zool. and Brindelle College, University of Toronto, Toronto, Ont., Canada.
Nervous tissue rich in myelin possesses high activity of CNP (EC 3.1.4.37). Consequently CNP activity has been accepted as a myelin index.

Previous findings from our laboratory have established that the distribution spectrum of axon diameter in the optic nerve of coldfish varies with temperature of acclimation. At 5°C there is a greater proportion of large axons greater than 0.9µm in diameter than at 25°C (cf. 71% and 22%). In order to relate these morphological observations to a biochemical assessment of myelin, CNP activity was determined in optic nerve and tectum; e.g. molar ratios of phospholipid phosphatidyl ethanolamine/phosphatidyl choline (PC), and galactolipid/PC. With elevation in acclimation temperature these ratios decrease in nerve and rise in tectum. An interesting observation is that the CNP activity/µmoles galactolipid ratio is lower at higher acclimation temperatures in all myelin-rich preparations (spinal cord myelin, brain myelin). As expected, the optic nerve possesses significantly higher CNP activity in the 5° fish compared with the 25°. It was found also that brain myelin has higher activities at the lower acclimation temperature. In the optic tectum, a tissue containing little myelin, there is no difference in the CNP activity withacclimation temperature. Other biochemical indices of myelination were determined in optic nerve and tectum; e.g. molar ratios of phos-phatidyl ethanolamine/phosphatidyl choline (PC), and galactolipid/PC. With elevation in acclimation temperature these ratios decrease in nerve and rise in tectum. An interesting observation is that the CNP activity/µmoles galactolipid ratio is lower at higher acclimation temperatures in all myelin-rich preparations but remains the same in the optic tectum.

These findings not only confirm the findings from morphological studies that the amount of myelin changes during temperature acclimation but indicate that there may also be a change in the composition. The significance of these findings will be discussed.
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PUTATIVE NEUROTRANSMITTER RECEPTOR SITES IN RAT HIPPOCAMPUS.

Dee Ann Matthews, Paul Salvaterra and Renee Foders*. Div. of Neurosciences, City of Hope Nat. Med. Center, Duarte, CA 91010.

The hippocampus is well suited for studies of synaptic transmission mechanisms in CNS by reason of its simple laminated organization, defined connections and variety of neurotransmitter systems. In order to study the quantitative relationship among various neurotransmitter systems in rat brain hippocampus, the levels of several putative neurotransmitter receptors were studied by radiolabeled ligand binding techniques. The levels and affinities of the various ligands used were determined by Scatchard analysis of binding isotherm data using crude membrane preparations. All ligands showed only a single high affinity site. Data is presented in the table below. Data were also analyzed by TTEST plots and all ligands had slopes near 1, indicating the absence of any cooperative interactions. Pharmacological characterization of the ligand binding sites indicated properties consistent with their identification as functional neurotransmitter receptors. The rank order of the total number of putative receptor binding sites (gabergic/cholinergic/adenergic) is in agreement with current estimates of the relative number of terminals contributed by each system. Supported by NIH NS 1216.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Putative Receptor</th>
<th>Bmaxa (fmol/mg)</th>
<th>Kp (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]Muscinol</td>
<td>GABA</td>
<td>20±9</td>
<td>5.47±9</td>
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<tr>
<td>[3H]quinolinylbenzilate</td>
<td>muscarinic-cholinergic</td>
<td>86±3</td>
<td>2.21±9</td>
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<td>[3H]dihydroergocryptine</td>
<td>α-Adrenergic</td>
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<td>[3H]hydroalprenolol</td>
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<td>67.8±3</td>
<td>2.72±9</td>
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<tr>
<td>[125I]Bungarotoxin</td>
<td>nicotinic-Cholinergic</td>
<td>43.2±3</td>
<td>1.19±9</td>
</tr>
</tbody>
</table>

a Values are derived from Scatchard plots of two sets of binding isotherm data using 6 different concentrations of ligand in triplicate.

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Mechanistically the mode of action of antidepressant drugs may be related to (1) increased neuronal stores of biogenic amines with resultant augmented synaptic concentrations at specific neurotransmitter via inhibition of oxidative deamination pathways (MAO), (2) inhibition of presynaptic monoamine uptake leading to increased extraneuronal concentrations at receptors and enhanced activity of neuronal circuits, (3) β-adrenergic receptor subsensitivity as indicated by a decrease in norepinephrine-induced cyclic AMP accumulation in clonal cell lines (Vetulani, Arch. Pharmacol. 293: 109, 1976) or cortical [3H]-dihydroalprenolol binding (Banerjee e al., Nature, 268: 455, 1977; Saral et al., Biochem. Pharmacol. 36: 205, 1987). None of these biochemical parameters were utilized to evaluate the mode of action of a series of selected antidepressant and psychoactive agents. Extremely weak in vivo inhibitory effects on rat brain mitochondrial MAO-type A or B were observed with HRP-197 and HP-505, both 3-aryl-spirobenzofuran2,3-diones, a 3-aryl-spirobenzothiophenone derivative, UK-5008 an indolylthiopiperidine, desipramine, nisoxetine and P76-2543, a novel heterocycle possessing antidepressant characteristics. As anticipated, desipramine showed potent substrate selective inhibition of MAO type B. The kinetics (Bmax and K) of [3H]-dihydroalprenolol binding were also studied following chronic administration of these same drugs (10 mg/kg, b.i.d.). After a 10-day treatment regimen all antidepressants resulted in decreased binding of [3H]-dihydroalprenolol binding. The effects of nisoxetine, clomipramine, noradrenergic agonists and antagonists were studied in order to elucidate the mechanisms by which antidepressants block the uptake of either norepinephrine, dopamine or serotonin into intact synaptosomal preparations. While present biochemical antidepressant tests in vivo have been in vitro, attempts were made to utilize the selectivity of uptake blockade of either norepinephrine, dopamine or serotonin into intact synaptosomal preparations. While the antidepressant activity of these drugs is in cell type and mode of action at the cellular level still remains to be elucidated.

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BRAIN CYCLIC NUCLEOTIDE LEVELS IN RATS SENSITIVE TO AUDIONERGIC SEIZURES. James L. Monsteroff, J. Kant, T. Daryl Hawkins and Robert H. Lenox. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington, DC.

The subjects were seventh generation Wistar-derived male rats selectively bred for sensitivity to audiogenic seizures (AS). Groups of adult AS-strain rats and adult control (C) male Wistar rats of equal size and from identical maintenance conditions were subjected to audiogenic challenge. None had ever previously been exposed.

Rats were placed singly in a metal grid chamber (size 33x24x13 cm) with a sound horn affixed to the top of the chamber. Auditory challenge was produced by an amplifier emitting white noise within a 17.0 - 14.0 kHz range at an intensity of 105 ± 1 db at floor level. Each sound exposure was 1 min in duration. Of the 15 AS rats challenged, five (33%) responded with wild running followed by clonic-tonic seizure. Five of the AS strain responded. Under similar testing procedures at three weeks of age, rats from the AS strain show a 5% incidence of clonic-tonic seizure. The effect of various agents from the control strain remained. Several attempts at clonic-tonic seizures at 3 to 52 weeks of age, no clonic-tonic seizures have ever been observed.

Separate groups of AS (N=6) and control rats (N=6) were sacrificed for biochemical studies. Average body weight in each group was 321 grams. Neither group was ever subjected to audiogenic challenge. Sacrifice was accomplished by a 5 second exposure to 2.5 KW microwave irradiation at 2450 MHz. Brains were dissected into 25 regions and assayed for cAMP and cGMP by fluorimetric assay. Brain regions assayed included lateral geniculate, nucleus accumbens, septal region, hippocampus, amygdala, medial geniculate, lateral geniculate, frontal cortex, and areas 2, 17, 24, 29, 41, and 51. The pineal and pituitary were also assayed.

In rats of the audiogenic seizure sensitive strain, cyclic AMP was elevated in the hippocampus, hypothalamus and vermis of the cerebellum.

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Changes in γ-AMINOBUTYRIC ACID RECEPTOR BINDING IN THE POSTMORTEM CAT CENTRAL NERVOUS SYSTEM. G. Keil Matheson and Godfrey Tunnicliff*. Evansville Center, Indiana University School of Medicine, Evansville, IN 47732.

Postmortem GABA receptor binding is becoming a useful measurement in the investigation of the role GABA in diseases of the nervous system. One of the problems of studying human postmortem brains is the lack of standardized procedures for their storage. This investigation was undertaken to determine if postmortem changes in GABA binding occur when brains are stored for different periods of time before the membranes are prepared. GABA receptor binding in fifteen regions of cat central nervous system was investigated immediately postmortem, and at twenty-four and seventy-two hours postmortem. A two-fold increase in binding was observed after twenty-four hours in the cerebellum, the sensorimotor cortex, the visual cortex, and the amygdala. Substantial increases were also noted in the thalamus, caudate nucleus, hippocampus, and hypothalamus. At seventy-two hours postmortem further increases in GABA binding were seen. Membranes from the pons and spinal cord did not exhibit appreciable changes in GABA binding. These findings suggest that interpretation of GABA binding data obtained from human brains that have not been treated in a similar postmortem manner should be made with reservation.

<table>
<thead>
<tr>
<th>Region</th>
<th>Hours Postmortem</th>
<th>HOURS POSTMORTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>263±34</td>
<td>72±9</td>
</tr>
<tr>
<td>Visual Cortex</td>
<td>136±19</td>
<td>32±7</td>
</tr>
<tr>
<td>Sensorimotor Cortex</td>
<td>130±21</td>
<td>31±5</td>
</tr>
<tr>
<td>Amygdala</td>
<td>14±33</td>
<td>32±19</td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td>110±15</td>
<td>166±22</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>80±18</td>
<td>131±16</td>
</tr>
<tr>
<td>Thalamus</td>
<td>72±9</td>
<td>116±13</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>71±10</td>
<td>94±14</td>
</tr>
<tr>
<td>Pons</td>
<td>26±8</td>
<td>34±9</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>24±5</td>
<td>32±7</td>
</tr>
</tbody>
</table>

These values are the means ±S.E.M. of GABA binding (f moles/mg protein). Each determination was done in triplicate. Three animals were used in each experiment.

A wide variety of neuropsychological and psychological studies in sensory systems have demonstrated a direct relationship between the magnitude of the response and the logarithm of the intensity of the stimulus. This relationship is known as the Weber-Fechner Law. In a recent study, the authors used a recently developed [3H]deoxyglucose method for measuring local cerebral glucose utilization (LCGU) to examine this relationship in the visual system of the rat.

The authors found that LCGU in the visual cortex and in the structures of the visual pathway, but this activity was confined to a narrower range of light intensity (0-7 lux), above which the metabolic response decreased with increasing intensity of stimulation. However, the results demonstrated that the response in energy metabolism in the visual system to variations in intensity of stimulation, like behavioral and perceptual responses, to the Weber-Fechner Law.


The olfactory sensory neurons which is followed by the process of degeneration and reconstitution of the olfactory neurons. The authors used immunohistochemical techniques (Monti Graziadei et al; J. Histochem. Cytochem., 1977) to observe the morphological changes of OMP content in the olfactory epithelium following axotomy. At five days survival time, the mucosa was almost completely devoid of OMP. In the olfactory nerve, all the immature elements were present, only a few immature elements were present. By the 12th day the neuronal population had reconstructed and contained only young maturing neurons. A few mitoses remained, the peak having already occurred between the 8th and 11th days (Monti Graziadei and Graziadei, J. Neurocytol. The number of OMP-positive cells decreased considerably in a few scattered neurons and in very fine bundles of axons leaving the epithelium. At 30 days survival time, the content of OMP had increased and larger bundles of positive fibers were observed in the epithelium. At 64 days the operated side was indistinguishable from controls, both in olfactory and peroxidase-antiperoxidase stained sections.
THE ACTIVATION OF ADENYLATE CYCLASE AND PHOSPHODIESTERASE IN C-6 GLIOMA CELLS BY THE CALCIUM-DEPENDENT REGULATOR PROTEIN. Jon R. Norman, Friedrich Miecher-Institute, P.O. Box 910, CH-4002 Basel, Switzerland.

C-6 astrocytoma cells provide a homogeneous cell type for studying the role of Ca++ in regulating the enzymes involved in cyclic nucleotide metabolism. Adenylate cyclase and cyclic nucleotide phosphodiesterase activity in this cell line, when subjected to the CAMP synthesis and degradation, respectively, are present in different isozyme forms in C-6 glioma cells. The calcium-dependent regulator (CDR), a calcium-binding protein, is also present in C-6 cells and this protein, when complexed with Ca++, can bind to and activate one form of both adenylate cyclase and phosphodiesterase. A procedure has been developed for the partial purification of CDR-sensitive phosphodiesterase using affinity chromatography with CDR attached to sepharose. The CDR-sensitive form of phosphodiesterase will bind to the CDR-sepharose column in the presence of Ca++ and will elute from the column in the presence of EGTA. This procedure utilizes a preliminary chromatographic step on DEAE cellulose which removes CDR and modulator-binding protein from phosphodiesterase. The modulator-binding protein is an inhibitor of CDR-stimulated phosphodiesterase and will copurify with CDR-sensitive phosphodiesterase in other CDR affinity chromatography procedures. This two-step procedure allows the partial purification of CDR-sensitive phosphodiesterase and yields a sufficient number of C6 cells to allow the final purification of the enzyme by sephacyr S-200 chromatography. The activation of CDR-sensitive phosphodiesterase under physiological ionic conditions is presently being investigated in addition to an analysis of the subunit structure of this enzyme by SDS gel electrophoresis. A similar purification procedure for adenylate cyclase is being developed.

IN VITRO INHIBITION OF ACETYLCHELINOSTERASE BY SEROTONIN AND BY AN UNIDENTIFIED SEROTONIN-DERIVED COMPOUND(S) AS STUDIED AT PHYSIOLOGICAL SUBSTRATE LEVELS. B. Osterfeld-Wenig, J.A. Simon, L. Chang*, and W.H. Aprison. The Institute of Psychiatric Research Depts. of Biochemistry, Pharmacology and Psychiatry, Indiana U. School of Medicine, Indianapolis, 46223.

Inhibition of acetylcholinesterase activity (ACHE) by serotonin (5-HT) and by an unknown compound(s) derived from 5-HT was studied using a series of physiological concentrations of acetylcholine (ACh), as substrate (10^-4 M). Freshly prepared solutions of 5-HT (10^-7 M) were found to inhibit ACHE from rat stomach as well as to inhibit the enzyme in a controlled batch. The degree of inhibition was dependent on the ratio of inhibitor to enzyme as well as on preincubation of the enzyme with inhibitors. The inhibition of ACHE by compound(s) was analyzed as a function of the duration of the enzymatic reaction. Inhibition of ACHE by freshly prepared solutions of 5-HT was found to be concentration-dependent in the range of 10^-10 to 10^-4 M 5-HT with resulting inhibition of approximately 30%. Kinetic analysis of the compound inhibition indicated it to be of a noncompetitive nature with a K_i of approximately 0.2 mM. When 5-HT solutions were exposed to air before incubation and 5-HT, a greater inhibition of ACHE was observed than when freshly prepared solutions were employed. Inhibition obtained with "exposed 5-HT" solutions also revealed noncompetitive inhibition; however, the K_i was reduced by 50% (0.1 mM). The enhanced inhibitory potency of the "exposed 5-HT" solutions suggested the formation of some additional compound(s) derived from 5-HT under conditions of exposure to light and air. Analysis of these solutions employing the techniques of high performance liquid chromatography and thin layer chromatography indicated the presence of the unknown substances exposed 5-HT. Exposing the time course of production of the unknown compound(s), a progressive increase in peak height was observed. A concomitant increase in inhibition of ACHE was also observed 12 days after exposure. The maximum peak height of the unknown corresponded to approximately 5% of the 5-HT peak height. At this time, the inhibition of ACHE by the unknown compound was a better inhibitor than 5-HT. Preliminary factors which influence the production of this unknown inhibitor indicated that (a) each oxygen and temperature enhanced this production, and that (b) the two factors can act synergistically. The inhibitory effects of 5-HT and/or "exposed 5-HT" on ACHE appeared to be highly specific and no effects were observed on the cholinergic parameters such as high affinity uptake of choline, choline acetyltransferase or muscarinic receptor binding. The process by which the unknown in this model might be reduced in high yields becomes important to elucidate from both the biological and clinic point of view. (Grant MH3225-20 and Fogarty Fellowship (BON))

RESPIRATION IN DISASSOCIATED NEONATAL RAT BRAIN PRIMARY CELL CULTURES. James E. Olson and David Holtzman. Depts. of Neurology and Pediatrics, Stanford Univ. School of Medicine, Stanford, CA.

Astrocytes are believed to possess certain unique properties of metabolism and respiration important in brain physiology. We have utilized the methods of neurobiology and transmembrane exchange to obtain a relatively pure preparation of astrocytes in culture. This affords the possibility of measuring the respiration in an intact astrocyte. astrocyte, unconfounded with neurons or endothelial components.

Neonatal rat cortical brain tissue was dissociated by treatment with trypsin followed by filtration through an 80 µm mesh. The cells were grown to confluency (two to three days) in culture, after culturing, the nutrient medium was replaced in the culture dish and the cells were stained positively for the astrocyte-specific glial fibrillar acidic protein (GFAP). At various times after culture, chemically unconfounded with neurons or endothelial components.

Cultured astrocytic cells were grown to a monolayer were removed from the culture dish with a mild trypsin exposure, and placed in a sealed oxygen chamber at 37°C for polarographic measurement of respiration. The oxygen consumption was constant over a wide range of nO_2. The average basal rate was 148±3 % of the basal rate. After a period of 15 minutes, it was determined that the basal rate could remain this. The maximal MnP-activated rate was 150% of the basal rate. The rate of treated cells compared to 148±3 % in untreated cells (n=11). With MnP-activated, MnP-activated cells and MnP-activated cells to produce MnP-activated cells. With MnP-activated, MnP-activated cells and MnP-activated cells to produce MnP-activated cells.

The results will be compared with observations on brain slices and isolated brain mitochondria.
**NEUROCHEMISTRY**

**EFFECT OF INORGANIC LEAD ON RAT BRAIN MITOCHONDRIAL RESPIRATION IN VITRO.** J. J. O'Neill, B.B. Naiman*, L. Epstein*, E. O'Neill*, Dept. of Pharm., Temple Univ. Medical School, Philadelphia, Pa. 19140. Inorganic lead, added to the diet of suckling rats in high doses has been shown to produce a cerebellar encephalopathy, and to inhibit the respiration of subsequently isolated cerebellar and, to a lesser extent, cerebral mitochondria (Holtzman and Shen Hsu, Pediat. Res. 10:70-76, 1976). Little data exists on the in vivo effects of lead on mitochondrial function in the brain of naive rats, largely due to the problems of Pb2+ chelation and precipitation by EDTA and inorganic phosphate.

**Mitochondrial isolation from rat brain (cerebellum removed) by a modification of the method of Nicklas and Clark (JBC 245:4724, 1970).** The mitochondrial pellet was washed twice with an EDTA free medium containing 0.025 M potassium phosphate, 10 mM EDTA, pH 7.4, and suspended in the same medium. Omission of EDTA from the incubation medium produced a large decrease in the respiratory control ratio with pyruvate (10 mM) plus malate (2.5 mM) as substrates. Pb-acetate (50 μM) when added following the addition of substrate but 2 min prior to the addition of inorganic phosphate (500 μM) produced a 25% inhibition of State 3 respiration on the addition of ADP, 0.5 mM Pb-acetate produced a 25% inhibition of State 3 respiration. The amount of lead bound to the mitochondria was determined by anodic stripping voltammetry following digestion of the filter in nitric/sulfuric perchloric acid (24:1:24). The present experiments demonstrate a dose-response relationship between lead and inhibition of mitochondrial respiration. (Supported by EPA contract 68-03-2381).

**POTASSIUM INDUCED SWELLING OF A GLIAL ENRICHED PREPARATION: THE FROG FILUM TERMINALE.** D. J. O'Neill, B. R. Melamed*, D. Epstein*, K. O'Neill*, Div. of Neurosurgery, Marine Biomedical Institute, and Deps. of Neurology, Physiology & Biophysics and Human Biol, Chem. & Genetics, University of Texas Medical Branch, Galveston, TX, 77555.

The phenomenon of potassium (K+) induced swelling of neural tissue has to date been largely studied in a variety of preparations containing both neurons and glia. The glial component of the preparation (FT), which contains largely glia and myelinated fibers, offers a unique opportunity to study the same aspects of K+ induced swelling in a normal glial preparation devoid of neurons. In these experiments the extracellular space was measured by incubating excised portions of FT in the presence of 3H-insulin in an oxygenated bicarbonate buffered frog ringer at 25°C containing various concentrations of K+ (Na+ was covarred to maintain isotonicity). Parallel experiments were performed using excised spinal cord (SC), which contains both neurons and glia. Results (extracellular space, ECS) are expressed in micrometers per mg. wet weight tissue.

K+ FT, which has been shown to have a lower cellular density than the SC on the basis of DNA measurement, has a correspondingly larger ECS (58%). Increases in the extracellular K+ greater than 30 mEq result in shrinkage of FT. The magnitude and rate of swelling are similar in both preparations. The enzyme, carbonic anhydrase, has been implicated in K+ induced swelling in mammalian neural tissue. However, addition of acetazolamide, an inhibitor of carbonic anhydrase, does not change the extent of K+ induced swelling in the frog FT or SC. Furthermore, the range of K+ concentrations which induces swelling is far higher in the frog than those previously reported for mammalian preparations. In other experiments, frog FT and SC are shown to have relative amounts of cells and extracellular activity. Taken together, these data indicate that K+ induced swelling in amphibian neural tissue, and particularly in glia, is mediated by carbonic anhydrase independent mechanisms, in contrast to those seen in mammalian brain. In the specific experiments of K+ induced swelling of amphibian neural tissue is under further investigation.

Supported by DHN Grant, A Center for Study of CNS Injury, NS-07377-09.

**ILEOCHOLIC EVIDENCE FOR INDUCTION OF CHOLINE ACETYLTRANSFERASE IN PREOPTIC AREA OF RAT BRAIN EXCITED BY ESTRADIOL.** Jong H. Park, Victoria R. Luine, Tong H. Joh, Bruce R. Judson and Donald J. Reis, Laboratory of Neurobiology, Cornell Unive. Medical College, and Rockefeller University, New York, New York 10021.

The circulating ovarian hormone estradiol (E2) is accumulated and retained with regional selectivity in brain, notably in the mesial preoptic area and hypothalamic. These brain regions contain binding sites for the hormone in nuclear and cytosol fractions. In the preoptic area of ovariectoized (OVX) rats, E2 increases the activity of choline acetyltransferase (CAT) (Endocrin. 100:903, 1977), the enzyme catalyzing acetylcholine synthesis. We sought to establish by immunochromatography using a specific antibody to CAT (Nature 275:324, 1978), the presence of CAT activityexcited by E2 is due to an increase in enzyme molecules or to change in its catalytic activity. In the first experiment, thirty days after removal of ovaries and adrenals, female rats (Long-Evans) received estradiol benzoate (CB)(100μg/250g body weight, s.c.) dissolved in sesame oil or sesame oil alone. The rats were sacrificed 24h later, the preoptic area removed and CAT activity measured. E2 increased CAT activity to 125% of control (15.5 to 19.3nmol/mg protein/10min). In the second experiment, three groups of female rats (Sprague-Dawley C5 strain) were ovariectoized and 14d later received the following agents, were sacrificed 3h after the last injection; preoptic area exposed and assayed for CAT. The injection schedule and CAT activity were:

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure</th>
<th>Amount</th>
<th>Duration</th>
<th>CAT activity (nmol/μg protein/10min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>I</td>
<td>25μg</td>
<td>24h</td>
<td>125%</td>
</tr>
<tr>
<td>B</td>
<td>II, III</td>
<td>30μg</td>
<td>14d</td>
<td>100%</td>
</tr>
<tr>
<td>C</td>
<td>IV</td>
<td>30μg</td>
<td>14d</td>
<td>125%</td>
</tr>
</tbody>
</table>

This experiment showed that E2 increases CAT activity in preoptic area after chronic but not acute treatment. Immunochromatographic identification of CAT showed that the equivalence point was unshifted to the right by approximately 30μg, demonstrating that the increases in CAT activity are entirely attributable to increases in CAT protein. The study demonstrates for the first time that: (a) the activity of CAT, like catecholamine synthesizing enzymes can be increased by the accumulation of the enzyme protein (induction), and (b) estradiol induces CAT regionally in rat brain. (Supported by NIH grants BL1897, BI24025 and EP 12041).

**A COMPARISON OF CHRONIC PRENATAL AND POSTNATAL HALOTHANE EXPOSURE ON BRAIN MYELIN SYNTHESIS.** Patasius, P.R., Riger, R.M., and Wiggins, R.C. Dept. Neurobiology and Anatomy, Univ. Texas Medical School, Box 20708, Houston, TX 77025.

Long-Evans rats were exposed prenatally or postnatally to 0.5% halothane for 8 hours/day during the specified age interval. Synthesis of brain subcellular membrane proteins (myelin, nuclear, synaptic, mitochondrial, and microsomal) in various subcellular regions contain binding sites for the hormone in nuclear and cytosol fractions. In the preoptic area of ovariectoized (OVX) rats, E2 increases the activity of choline acetyltransferase (CAT) (Endocrin. 100:903, 1977), the enzyme catalyzing acetylcholine synthesis. We sought to establish by immunochromatography using a specific antibody to CAT (Nature 275:324, 1978), the presence of CAT activity excited by E2 is due to an increase in enzyme molecules or to change in its catalytic activity. In the first experiment, thirty days after removal of ovaries and adrenals, female rats (Long-Evans) received estradiol benzoate (CB)(100μg/250g body weight, s.c.) dissolved in sesame oil or sesame oil alone. The rats were sacrificed 24h later, the preoptic area removed and CAT activity measured. E2 increased CAT activity to 125% of control (15.5 to 19.3nmol/mg protein/10min). In the second experiment, three groups of female rats (Sprague-Dawley C5 strain) were ovariectoized and 14d later received the following agents, were sacrificed 3h after the last injection; preoptic area exposed and assayed for CAT. The injection schedule and CAT activity were:

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<th>Exposure</th>
<th>Amount</th>
<th>Duration</th>
<th>CAT activity (nmol/μg protein/10min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>I</td>
<td>25μg</td>
<td>24h</td>
<td>125%</td>
</tr>
<tr>
<td>Group B</td>
<td>II, III</td>
<td>30μg</td>
<td>14d</td>
<td>100%</td>
</tr>
<tr>
<td>Group C</td>
<td>IV</td>
<td>30μg</td>
<td>14d</td>
<td>125%</td>
</tr>
</tbody>
</table>

This experiment showed that E2 increases CAT activity in preoptic area after chronic but not acute treatment. Immunochromatographic identification of CAT showed that the equivalence point was unshifted to the right by approximately 30μg, demonstrating that the increases in CAT activity are entirely attributable to increases in CAT protein. The study demonstrates for the first time that: (a) the activity of CAT, like catecholamine synthesizing enzymes can be increased by the accumulation of the enzyme protein (induction), and (b) estradiol induces CAT regionally in rat brain. (Supported by NIH grants BL1897, BI24025 and EP 12041).
HEXOSAMINIDASES IN HUMAN BRAIN AND TUMORS OF THE NERVOUS SYSTEM.

Robert B. Ramsey. Department of Neurology, Saint Louis University, St. Louis, Mo. 63104.

Total hexosaminidase activity of nervous system tumors has been found to be consistently greater than that of normal brain. Studies utilizing tissue homogenates for determination of apparent Km values have suggested that hexosaminidase derived from tumor tissue differs from that in brain in other respects as well. Since this enzyme may serve as a key enzyme in maintaining the neoplastic condition, we have sought to further characterize the hexosaminidase of nervous system tumors. Using a resuspended ammonium sulfate derived from 37,000 x g supernatant of a homogenate of lymphophiliated brain and tumor preparations, it could be shown that initial gel filtration on G-150 Sephadex yielded two peaks of enzyme activity; the lesser peak of activity was associated with the void volume (peak I), the greater activity with an approximate MW of 135,000 (peak II). In normal brain tissue, peak I represented 35% of the total activity in grey matter, and 30% of the total activity of white matter. In comparison to normal white matter, the void peak was significantly reduced (P<.01) in preparations of glioblastomas. Other tumors which have been analyzed (meningealomas, astrocytomas and tumors metastatic to the nervous system) also demonstrate a reduction in peak I. DEAE-cellulose chromatography of the two peaks derived from gel filtration demonstrated that peak II was dominated by hexosaminidase A in normal white and grey matter. Isozyme A constituted 85% of the total eluted activity of human grey matter and 92% of white matter activity. Only 6% of total eluted activity of grey matter was isozyme B. It represented 6% of the white matter activity. The remainder of the activity was derived from isozyme I. In normal brain tissue, peak I contained only isozymes B and A. Analyzing glioblastomas samples in the same manner, it was found that DEAE-cellulose chromatography of peak II yielded a significant (P<.01) increase in the relative content of the B isozyme, compared to normal in the relative content of the B isozyme, compared to normal White matter, and a significant (P<.01) reduction in the relative activity of isozyme A. A significant increase in the relative activity of isozyme B in peak II was observed in the chief tumors. Peak activity from these tumors could not generally be eluted from DEAE-cellulose using a 0-3M NaCl gradient. Comparable isozyme patterns were observed in tumors preparations which contained only 5-nitrophenyl N-acetyl-β-D-galactosaminide as substrate. Although specific properties of the isozymes derived from these tumors have yet to be defined, it is already obvious that the alterations in total enzyme activities also reflect changes in isozyme content as a result of neoplasia.

GABA LEVELS IN CEREBROSPINAL FLUID OF NEUROLOGICAL PATIENTS.


Recently we have developed a simple and sensitive assay for the measurement of GABA in fluids and tissues by high performance liquid chromatography (Proc. International Soc. Neurochem. 6, 444, 1977). The only sample preparation required is deproteinization and usually 100 µl of CSF is all that is required for a reliable determination of GABA. We have measured GABA CSF levels in patients with various neurological disorders as well as in a few control specimens. The control values, around 26-150 µM with occasional high values and are in general agreement with those reported by Enna et al. (Arch. Neurol. 34, 683, 1977).

GABA CSF levels in patients with myoclonus, stroke, dystonia and other miscellaneous neurological disorders ranged from 50-600 µM, without any recognizable pattern. From the results obtained thus far, low levels of CSF GABA are not necessarily limited to patients with Huntington's chorea or Parkinson's disease.

VARIOUS ASPECTS OF L-DOPA DECARBOXYLASE IN THE BRAIN.

K. S. Rajan, M. Robinson*, IIT Research Institute, Chicago, IL 60616, and John M. Davis, Ill. State Psych. Inst., Chicago, IL 60612.

Consistent with a metal chelation approach, in vivo studies have been carried out to investigate the activity of the L-DOPA-decarboxylase on the 14C-L-DOPA chelates of Cu(II) containing equimolar amounts of citric acid, dipyridyl and ATP. Warburg-technique was used to determine the 14CO2 released from the decarboxylation reaction. The chelate substrates investigated were: 14C-L-DOPA-Cu(II)-citric, 14C-L-DOPA-Cu(II)-dipyridyl, 14C-L-DOPA-Cu(II)-ATP (1:1:1) and 14C-L-DOPA-Cu(II) (2:1). In the range of concentration of 0.1 to 0.4 µM of the substrates, a substantial inhibition of the decarboxylase activity was observed, i.e. that the decarboxylase activities of the chelate substrates are examined in the light of the metal L-DOPA binding strengths. The potential clinical significance of this approach to Parkinsonism is discussed.
SPECIFICITY OF ASSOCIATION OF A CACGAG ATTIC WITH CHOLINERGIC SYNAPTIC VESICLES FROM TORPEDO ELECTRIC ORGAN. John E. Rothlein and Stanley M. Parsons (SPON: Harry J. Cornfield). Marine Science Institute and Department of Chemistry, University of California, Santa Barbara, CA 93106.

Purified cholinergic synaptic vesicles from the electric organ of Torpedo californica have been subjected to analytical scale separation techniques not utilized in the isolation procedure, and the ATTase activity has been quantified. Most of the ATTase activity migrated with the vesicles. Sensitivity of the ATTase activity to 16 potential inhibitors also was determined. Most of the ATTase activity was inhibited by low concentrations of 4-chloro-7-nitrobenzoxazolinedione (NBQ-CI) and dicyclohexylcarbodiimide (DCCD), but not by a water soluble carbodiimide. Chlorpromazine also inhibited ATTase activity significantly. The close association of the ATTase with the vesicles and the pattern of inhibition obtained provide further support for the authentic presence of a membrane bound CACGAG ATTase in the cholinergic synaptic vesicle.

THE ECC SYNDROME: POSSIBLE CHOLINERGIC INVOLVEMENT IN AN ANIMAL MODEL OF HYPERKINESIS. A.C. Sconzert, B. Haber, and S. Cabay. Marine Biomedical Institute, UTMB, Galveston, TX 77550, V.A. Hospital, Brockton, Mass.

Eighteen unipolar depressed (drug free) patients and ten normal controls were voluntarily hospitalized for six days and five nights. Under control conditions, the following laboratory biochemical measurements were monitored. Using standard biochemical procedures the following measurements were made on blood drawn after a 12 hour fast on days two, three, and five: (a) plasma levels of free and total tryptophan, (b) twenty other amino acids, (c) choline, (d) cortisol and (e) free fatty acids. Total protein, fat, and carbohydrates consumed with each meal were measured by a nutritionist. Each patient was rated daily for severity of depression using Hamilton and Beck questionnaires. In general, there were no significant effects of dietary protein, fat or carbohydrate on any of the plasma biochemical measurements performed. It was noted that although these groups consumed the same amounts of protein and carbohydrates, depressed patients consumed significantly higher amounts of fat. No differences were noted in the levels of plasma metabolites between the two groups except for tyrosine and phenylalanine which were significantly higher in depressed patients when expressed as the mean ± S.D. Thus tyrosine levels in the depressed group were 60.4 ± 9.4 nmol/ml and 51.3 ± 12.9 nmol/ml for controls. P < 0.01. Depressed patients were 56.0 ± 12.2 whereas controls were 47.6 ± 8.2. Significant differences (p < 0.04 and p < 0.06, respectively) were obtained using a student t-test based on the sum of the blood measurements. There were no differences in the ratios of either free or total tryptophan to the sum of the five competing amino acids (valine, leucine, isoleucine, phenylalanine and tyrosine). These neutral amino acids have been shown to block the uptake of tryptophan into the CNS. The data on ratios follow: Free tryptophan/free total tryptophan: 1:1.5. The depressed group was 1.024 ± 0.005; total tryptophan/ratio: depressed patients 0.089 ± 0.02, control 0.101 ± 0.03. Further, these ratios showed no significant differences in both groups irrespective of the concentrations of either free fatty acids and cortisol in plasma or dietary amounts of protein, fat and carbohydrates in the diet. The increased levels in the two compartments of neutral amino acids tyrosine and phenylalanine found in depressed patients suggests that tryptophan transport into the CNS of these patients may be affected. (Supported in part by Indiana Dept. Mental Health 178-679-005 and Indiana Attorney Generals Fund, 1979).


**1409**

**PREFERENTIAL LOCALIZATION OF γ-Glutamyl Transpeptidase in GLIA.**

R.D. Shime, J. Hertz, J. DeVallia, and E. Baer. Marine Biomedical Institute, UHMB, Galveston, TX 77550, UCLA School of Med., Los Angeles, CA. 90024, Univ. of Saskatchewan, Canada.

γ-glutamyl transpeptidase (γ-GTP) is an enzyme which may have a role in transport of amino acids and peptides across biological membranes. High levels of γ-GTP activity found in the brain suggest that this enzyme may well have such transport functions within neural tissues. We observe that in the central nervous system levels of enzymatic activity are greatest in the capillaries, followed by white matter and are significantly lower in gray matter. The higher levels of γ-GTP activity in white matter suggest a preferential association of γ-GTP with glia. To determine the cellular localization of γ-GTP in neural tissues we have employed cultured cells of neuronal and glial origin as models of neurons and glia in vivo. γ-GTP activity was measured using a highly sensitive fluorometric assay which employed the synthetic substrate 7γ-glutamylamido-4-methyl coumarin and high pressure liquid chromatography or fluorescent spectroscopy. Enzymatic activity in clonal cell lines of glial origin uniformly expressed high water activity than did a variety of non-neuronal cell lines. Furthermore the γ-GTP activity of normal non-transformed rodent astrocytes and oligodendrocytes was also higher than neuronal levels. No significant difference in γ-GTP activity was found between the normal astroglia and oligodendroglia. Taken together this data strongly suggest that the bulk of CNS γ-GTP activity is primarily associated with astroglia, oligodendroglia and CNS capillaries.

Supported by Welch H-504, PHS Grant NS 1255, NCI Grants CA 8877 and CA 11701, ESDA contract EV-76-C-03-0012 and MRC Grants DC 120 and NT 597.

**1411**

**ISOENZYME FORMS OF ACETYLCHOLINESTERASE IN RAT AND MOUSE TISSUE.**


Acetylcholinesterase (AChE) is present in rat skeletal muscle in at least three isoenzyme forms identified by sucrose density gradient centrifugation as 4S, 10S and 16S. During development, the 16S form has been called the endplate specific form as it is absent from non-innervated regions of the muscle and disappears from endplate regions after denervation. We have studied the pattern of γ-GTP activity in various regions as well as discrete regional concentrations in post mortem tissues. At 14 days after birth the 16S form is found in the brain, EDL and T-597. These results indicate that the 16S form is not restricted to endplate regions of skeletal muscle. (Supported by NHI Grants NS 11855 and NS 14304. W.S.B. is a recipient of Research Career Development Award NS 00119 from NIH.)

**1410**

**ACTIONS OF LEAD (Pb) ON GABAERGIC NEUROTRANSMISSION: DISPARITIES BETWEEN IN VITRO AND IN VITO EFFECTS.**

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Exposure to Pb in vivo produces convulsions and, at lower levels of exposure to sensory and motor disorders. The effects may be produced by chronic exposure of rats from birth (to 10 mg Pb acetate/kg) or by exposure of the neonatal to Pb at reduced concentrations of Pb acetate (7.58 mg/kg) for 3 days. Rods producing convulsions are decreased and duration of clonus is increased. In both Pb-exposed groups 4S Pb rats were chelated on an equimolar basis with pronic acid or bicuculline. Neurochemical studies were done on synaptosomes and synaptic membranes prepared from caudate (CA), cortex (CR), cerebellum (CB) and substantia nigra (SN) of acute and chronic Pb rats. Ne-dependent affinity 125I-GABA uptake, release of 3H-GABA (in the presence of 5 mM or 35 mM Ca2+), and Ne-dependent specific 3H-GABA binding were studied. In Pb rats, GABA uptake was inhibited in CB, CR, and SN. Kinetic analyses demonstrated that in these regions Vmax was reduced and that significant effects on Km. Release of GABA was also inhibited under conditions of spontaneous and Et-instilled stimuli. No changes were observed in any region for Na-dependent specific localization of the 16S form of GABAergic function, which may reflect rather a selective destruction of GABAergic function. In vivo, Pb in vitro has no effects on GABA uptake, release, or receptor binding, even when long exposure times are used. The discrepancy between in vivo and in vitro does not suggest that the actions of Pb in vivo on GABA uptake and release may result from indirect effects of Pb on other neurotransmitter systems or from the action of a toxic metabolite. In vivo, Pb may decrease CNS acetylcholine release and increase CNS dopamine release (Life Sci 1977: 20:309). A positive toxic metabolite affecting GABAergic function may be aminolevulinic acid (ALA), concentrations of ALA in tissue and plasma are greatly elevated after Pb exposure in vivo. However, ALA in vitro (as high as 1 mM) has no effects on synaptosomal GABA uptake or release. PB inhibits GABA receptor binding but only weakly (IC50 = 1 mM). The mechanism of action of Pb in vivo on GABAergic function are at present still unclear.
EVIDENCE FOR NEURONAL SYNAPSES ON THE BASEMENT MEMBRANE OF CEREBRAL CAPILLARIES. R.L. Siddith, K.E. Savage*, P.S. Baug*, J.S. Crawford*, and H.M. Eisenberg. Division of Neurosurgery, Shriners Burns Institute and Department of Surgery, University of Texas Medical Branch, Galveston, Texas 77550.

While innervation of large cerebral vessels by adrenergic and cholinergic nerve fibers have been shown, similar innervation of the cerebral microvasculature has not been demonstrated. In this study isolated cerebral microvessels were examined for presence of neuronal synapses. The microvesseles were isolated from the cerebral cortex of mature Sprague-Dawley rats by a process of serial filtration and differential centrifugation. The isolated contained numerous capillary segments and few segments of small arterioles and venules.

Transmission electron microscopy showed that the capillary segments were circumferentially complete, the endothelial cells being surrounded by an intact basement membrane. Electron dense areas containing numerous uniform vesicles were found external and adjacent to this membrane. Morphologically these areas were similar to neuronal terminals found on larger vessels. Using histochemical techniques the presence of monoamine oxidase was found in association with capillaries and using radioenzymatic assay techniques the microvessele isolate was shown to contain this enzyme.

These studies show that electron dense bodies present on the basement membranes of cerebral capillaries have the appearance of neuronal synapses and that the enzyme monoamine oxidase is present in this region. The microvesseles were isolated from the cerebral cortex of mature Sprague-Dawley rats by a process of serial filtration and differential centrifugation. The isolated contained numerous capillary segments and few segments of small arterioles and venules.

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It has been suggested that in the neuroepithelia of warm blooded animals the demand for protons and bicarbonate requires the presence of the enzyme carbonic anhydrase (CA). Thus the choroid plexus of the brain exhibits a high CA activity. In contrast, the choroid plexus of a cold-blooded species such as frog is devoid of the enzyme. To test the idea of a relationship between body temperature and CA activity we have measured the CA activity in a number of preparations from different brain regions with emphasis on the enteric nervous system structures. In nervous tissue CA is specific to neurons and absent from neurons. We observe that the general polikloithems exhibit much lower CA activity than do comparable tissues in homeotherms. There are exceptions to this observation, however. The blood of poliklothems has quite appreciable CA activity - almost 14,000 U/mg wt for the saltwater catfish. The lower brainstem (LBS) of the saltwater catfish exhibits as much CA activity as does LBS of the rat but in a ratio more than present in LBS of the cat, for example (103, 1602 and 906 U, respectively). An interspecies comparison of nervous system CA activity shows that for each species retina and neuroglia are enriched in CA activity and absent from neurons. We report here on CA activity in platelets and aorta. Platelet and aortic CA activity in homogenates of both CNS and PMS (myenteric plexus). This protein is enriched in brain synaptic vesicles. When enteric neurons were stimulated by serotonin binding proteins are released by a Ca2+-dependent mechanism. The protein binds newly synthesized serotonin; serotonin binding is inhibited by reserpine. We now report the properties of serotonin binding proteins in the brain. We have observed that centrifuged rat brain drawn by cardiac puncture, was frozen and thawed, and the resulting suspension was centrifuged (10000 x g, 60 min) to obtain the high speed supernatant. The binding of serotonin to the protein in this supernatant was highly specific (92%), dependent on Fe2+, trypsin sensitivity, and partially heat stable (42% decrease of activity at 100°). Two proteins with serotonin binding capacity were present: a glycoprotein and albumin. Both were also present in platelets sedimented from canine plasma. They were purified using (NH4)2SO4 fractionation, Sephadex sieving chromatography and affinity column chromatography. The two proteins differed in most characteristics. However they showed two similar properties: sensitivity to trypsin (85% loss of binding activity with 0.2 µg trypsin/ml at 20° for 20 min) and enhanced binding (5 to 10 fold) in the presence of total brain 10 (25 µg/lipid/25 µg protein). The proteins exhibited several differences: 1) migration on 5% acrylamide gel of the complex [protein-Fe2+, trypsin] was slower in canines than in rats; (B) 2) heat stability (100°, 15 min); glycoprotein unstable, albumin stable; 3) molecular weight (305 gels): glycoprotein 100,000; albumin 64,000. 4) carbohydrate reaction: glycoprotein stains strongly with Schiff's reagent after periodate oxidation; albumin, no reaction. 5) binding capacity and binding constants: glycoprotein about 25 µmole/mg; albumin 0.12 µmole/mg protein, two dissociation constants K1=2.0x10-9 and K2=5x10-10 M. The properties of both the glycoprotein and albumin are different from the characteristics of the serotonin binding protein of brain. It appears from these results that the storage form of serotonin in platelets is different from that of the brain.

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It has been suggested that the relationship between requirement for protons and bicarbonate and body temperature is not straightforward. A functional relationship between body temperature and CA activity would be supported by a decrease in CA activity in enteric neurons. Our data suggest that the relationship between requirement for protons and bicarbonate and body temperature is not straightforward. A functional relationship based on ion movement and acid-base balance might explain our observations. As for the nervous system distributions, it might be tempting to speculate that structures central to the functioning of the animal may require or regulate extracellular ion concentrations and therefore such regions require greater CA activity.

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Brain microsomes and synaptosomes were prepared from placebo controls and from mice pretreated by intracerebrally with carbamylcholine (CC) (4 µg/grain). When the membrane samples (app 800 µg protein) were incubated with labeled oleoylCoA (0.1 uCi in 5 nmoles), a large portion of the label was hydrolyzed by the microsomes. The carbamylcholine (CC) pretreatment increased the release of oleoylCoA when it was added in the presence of free fatty acids, but a small portion of the oleoyl group was transferred and subsequently incorporated into the phospholipids. The acyl-CoA hydrolase activity released from CC-stimulated mice as compared to controls. CC-induced convolution gave rise to a 3-fold increase in oleoyl transfer to diacyl- glycerophosphocholine (DGPC). The most rapid increase was observed during the initial 5 min after onset of convolution. Among other phospholipids in synaptosomes, the diacyl-glycerophosphoinositols (GPI) also indicated an increase in acylation (app 1-fold) with respect to CC-induced convolution, but the effect was not readily discernable due to low incorporation activity. The increase in acylation activity was persisting only to synaptosomal phospholipids, suggesting that specific types of lysophosphatidylglycerols were released in synaptosomes during CC-induced convolution. Calcium (4 µM) inhibited acyl-CoA hydrolase activity by app 25% but enhanced the acyl transfer into phospholipids, especially diacyl-GPC and diacyl-GPE (ethanolamine). The calcium-enhanced acyl transfer to diacyl-GPC and diacyl-GPE in synaptosomes was further stimulated during CC-induced convolution. Some increase in acylation of phospholipids due to calcium was observed in nervous tissue fractions. The increase in the loss of transfer to diacyl-GPS (serine) was most dramatic, giving a 3-fold difference with respect to CC-induced convolution. Among other phospholipids, the acyl-CoA transfer to membrane is attributed to the release in lysophosphoglycerides due to a stimulation of the phospholipase A2. Apparently, the sensitivity of phospholipase A2 stimulated by CC-GPC and diacyl-GPC in synaptosomes and diacyl-GPS in microsomes is altered with respect to convolution. Results of this experiment have demonstrated for the first time the involvement of deacylation-reaction mechanism in membrane phosphoglycerides during CC-induced convolution. The increased deacylation activity with respect to brain stimulation was mediated through an unknown mechanism of interaction between calcium and phospholipase A2. (Supported in part by NS-12960 from NIH and BNS 76 24338 from NSF.)
EFFECTS OF 3,4-DIHYDROXYLYPEPTIDAMINE AND ITS QUINONE ON THE UPTAKE AND RELEASE OF \(^{3}H\)-CATECHOLAMINES IN A RAT BRAIN CRUDE SYNAPTOSOMAL FRACTION. Anthony D. Vanker, Stephanie J. Prevost, and Frank L. O'Brien*. Dept. of Biology and Chemistry, Georgia State University, Atlanta, Georgia 30303.

The inhibition of uptake of 6-OHDA (6-OHDA) on catecholamine uptake into presynaptic elements are well known. There is evidence that at least some of the effects of 6-OHDA are due to its oxidation to the corresponding quinone which undergoes nucleophilic reactions. Similar quinones can be formed from normal catecholamine metabolites such as 3,4-dihydroxyphenylalanine (DHPA).

We previously reported on the use of controlled potential coulometry as the means of oxidizing DHPE and the effects on the uptake of \(^{3}H\)-norepinephrine (\(^{3}H\)-NE) (Sci. Neurosci. Abst. 6, 104). Crude synaptosomal fractions of rat brain homogenates were isolated and mixed with a TES salts buffer (NEP).

Different portions were then used in each of the following experiments: 1) NEP + stirring + DHPE + electrolysis. 2) NEP + stirring + DHPE + electrolysis. The time interval for each experiment was 10 min. Immediately following each experiment, standard procedures were used to measure the uptake of \(^{3}H\)-dopamine (\(^{3}H\)-DA) into synaptosomes found in the NEP. DHPE itself exhibited a weaker but potent inhibition in an apparent competitive manner. This inhibition of \(^{3}H\)-DA uptake obtained with DHPE and its quinone is similar to but more pronounced than that reported previously for \(^{3}H\)-NE. In some experiments, a brain mince was incubated in the TES salts buffer with either \(^{3}H\)-DA or \(^{3}H\)-NE to 'preload' presynaptic elements prior to homogenization and differential centrifugation. The preloaded fraction was re-suspended and treated as before to determine whether DHPE or its quinone act as releasing agents. DHPE did not cause the release of \(^{3}H\)-DA or \(^{3}H\)-NE at any of the concentrations used in the uptake study. The quinone was a weak releasing agent (25% for both \(^{3}H\)-DA and \(^{3}H\)-NE) only at the highest concentration used. Electrochemical data indicated substantial secondary oxidation reaction occurring in the presence of synaptosomes. (Supported in part by NS-14330).


Inactivation of tryptophan hydroxylase from rat midbrain was found to occur at 37°C in the presence and absence of calcium. A variety of non-protein-linked and other compounds. Control studies indicate a multiphasic inactivation characterized by a rapid initial loss with half time of 15 minutes followed by a slower, calcium-dependent loss. After 15 minutes, the rate of inactivation declined until stability of 40-50% of the initial activity was achieved at about one hour. The inactivation of tryptophan hydroxylase in the presence of calcium was measured at 100, 250, and 500 nmoles of calcium chloride (500, 1, 0.25, 1.0 µM), tetrabehydropyrroboxin (0.1 µM), reduced nicotinamide adenine dinucleotide (0.1 µM), dithiothreitol (0.1 µM), ethanol (1.0%), and phenylmethyl sulfonyl fluoride (0.1 µM); however, the effects of combinations of the above were not studied.

In the presence of greater than 200 µM calcium, loss of activity was rapid and followed classical first-order kinetics. At low and intermediate levels of calcium (200-800 µM), first-order loss of activity commenced after an initial period of stability lasting as long as 40 minutes. With higher levels of calcium, rapid loss of activity proceeded with no or minimal periods of stability. The range of calcium concentrations at which stabilization and destabilization are observed parallels that for the previously reported calcium activation of tryptophan hydroxylase (Life Sci. 16: 1583, 1975), suggesting that the molecular events underlying these three phenomena are intimately related, if not identical. The possibility of a calcium-activated protease, which mediates these events through an activating limited proteolysis followed by an inactivating proteolysis, is being examined. Recent preliminary preparations in the tissue for serotonin, when thawed, showed slightly higher initial activity but exhibited rapid first-order loss of activity when incubated at 37°C. These results indicate that the degree of activation of a proteolytic system may explain the time-dependent appearance of a destabilized enzyme population.

Preliminary observations indicate that tryptophan hydroxylase from rats housed at 30°C shows marked instability at 37°C when compared to the enzyme from rats housed at 22°C. We speculate that the stabilization of the system, proteolytic, or otherwise, sensitive to ambient temperature may represent a mechanism for the apparent role of the serotonergic system in temperature regulation.

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We investigated the permeability of the blood-brain barrier to cystine in autoradiographic studies using the rat carotid injection technique of Oldendorf in the rat. A mixture of a radioactively-labeled amino acid and \(^{35}S\)-cysteine was inhibited by 30% and 30%, respectively, of cystine, the reduced form, exists in equilibrium with cystine, the oxidized form. We measured the degree of blood-brain barrier penetration by cysteine and cystine using the carotid injection technique of Oldendorf in the rat. A mixture of a radioactively-labeled amino acid and \(^{35}S\)-H2O was injected into the carotid artery, the brain was decapitated at 5 or 15 seconds, and the brain dissected into 5 regions before counting. When \(^{35}S\)-cystine was injected, we found an initial delay until very close to the background values of this technique. The addition of non-radioactive cystine (200-600 µM) had no effect on the uptake of \(^{35}S\)-cysteine (25 µM) and \(^{35}S\)-methionine (25 µM) or of the basic amino acid, \(^{15}C\)-arginine (10 µM). In carotid injections using \(^{35}S\)-cysteine (10 µM), we found that this amino acid was readily taken up (BU) at 20%). When non-radioactive cycloleucine (4 mM) and alagone (4 mM) were added to the injectant, the uptake of \(^{35}S\)-cysteine was inhibited by 30% and 30%, respectively. Although cystine and cysteine are chemically related, their transport characteristics differ. Cystine can be considered a disulfide cysteine, we were unable to demonstrate a significant degree of blood-brain barrier transport at the concentrations tested. Cysteine, the sulfhydryl form of cystine, is related to cystine in structure and as an amino acid transport at the concentration of cysteine's total uptake. Another possible component of cysteine's uptake is the alanine-serine-cysteine (ASC) neutral amino acid transport system, which remains to be further evaluated.

(Supported by NIMH grant NS 13914 and the E.G. Schlierer Foundation.)
Acid chain near the COOH terminus appear to be covalently intact isolated protein, adjacent regions of the single amino residues at the COOH terminal end of the P2 protein. In the guinea pigs, rabbits and Lewis rats, CN3 proved inactive while Wannamaker). Departments of Neurology and Pathology (Neuropathology), Medical University of South Carolina, Charleston, S.C. P2 protein. CN3 is at the NH2 terminus, followed by CN1 with tryptophan at residue 8 enable us to order these peptides in the synthesis of NSE. These results show that the m-RNA coding for NSE contains a poly(A) sequence and that brain specific factors are not required for its translation. This is the first demonstration of the de novo synthesis of a neuron-specific protein which is 1% of the total soluble protein and whose biological function is known.

Three peptides denoted CN1, CN2 and CN3 are generated by CN2 produced disease in all three species. Experimental allergy, which in turn stimulate an auto receptor-linked adenylate cyclase, is also unlikely since α or β receptor blocking agents were found not to prevent Ca++ dependent cDPK activation. Also Ca++ would themselves produce an activation of cDPK. Unless some presently unknown agent, secreted in response to Ca++, is stimulating adenylate cyclase, a likely explanation for these results is a Ca++ interaction with calmodulin to modulate chromaffin cell Ca++ through sequential activation of adenylate cyclase and phosphodiesterase. The shapes of the time response curves at different agonist concentrations supports this conclusion. Supported by USPH NS 11061 and AG 00538.

When tested for their ability to produce disease in guinea pigs, rabbits and Lewis rats, CN3 proved inactive while CN2 produced disease in all three species. Experimental allergic neuritis (EAN) was produced in Lewis rats by peptide CNi as well, with this peptide being more active than CN2 in this species. NSE, which is neurotrophic in all species tested, is a disulphide-linked dipeptide accounting for approximately 18 residues at the COOH terminal end of the P2 protein. In the intact isolated protein, adjacent regions of the single amino acid chain near the COOH terminus appear to be covalently linked through a disulphide bond, which remains intact after cleavage of the two methionines resulting in CN2. The presence of this intra-chains disulphide bond in the native, membrane bound P2 protein and its effect on the disease-inducing properties of the P2 protein remain to be determined. Supported in part by the Veterans Administration and by NIH Grant No. NS 1867.

1427 P2 PROTEIN FROM BOVINE PNS MYELIN - NH2-TERMINAL SEQUENCE AND NEUROTIOTIC GENIC ACTIVITY. M. L. Weiss, D. J. Knight, S. Levine, P. H. Hoffmann, J. M. Powers and S. W. Brostoff (SPON: B. B. Wannamaker). Departments of Neurology and Pathology (Neuropathology), Medical University of South Carolina, Charleston, S.C. 29403 and V.A. Medical Center, Charleston, S.C.

When rat telencephalon slices are used to label protein with radioactive amino acids and these slices are subsequently fractionated by rate and density gradient centrifugation, two fractions, nuclei and blood capillaries, are found which have very high specific activities of radioactively labeled protein (White, 1979). Only the blood capillary fraction, however, appears to be transporting some of this newly synthesized protein to the other sites on the location system which in turn stimulate an auto receptor-linked adenylate cyclase, is also unlikely since α or β receptor blocking agents were found not to prevent Ca++ dependent cDPK activation. Also Ca++ would themselves produce an activation of cDPK. Unless some presently unknown agent, secreted in response to Ca++, is stimulating adenylate cyclase, a likely explanation for these results is a Ca++ interaction with calmodulin to modulate chromaffin cell Ca++ through sequential activation of adenylate cyclase and phosphodiesterase. The shapes of the time response curves at different agonist concentrations supports this conclusion. Supported by USPH NS 11061 and AG 00538.

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Recent work from this laboratory has shown that adrenal glucocorticoids control the steady-state levels of adrenal medullary phenylethanolamine N-methyltransferase (PNMT) by inhibiting the in vivo proteolysis of this enzyme. Further investigation disclosed that glucocorticoids were not acting directly, but through S-adenosylmethionine (SAM), an important constituent in the PNMT reaction. Hypophysectomy reduced the levels of glucocorticoids and of S-adenosylmethionine. This, in turn, resulted in a loss of stability of PNMT to in vivo proteolysis. SAM is a critical methyl donor in a variety of methylation reactions involving biogenic amines. Our current research shows that pineal hydroxyindole O-methyltransferase, like adrenal PNMT, is under glucocorticoid regulation. Hypophysectomy reduces the levels of HIOMT, while dexamethasone administration restores them. SAM, both in vitro and in vivo stabilizes HIOMT against proteolytic degradation, resulting in a restoration of enzyme levels. Thus the levels of two important biogenic amine methyltransferases, HIOMT and PNMT, are under glucocorticoid regulation through mechanisms involving SAM stabilization of the enzyme against proteolytic breakdown. (Supported by MH 25998, NSF PCM 78-14183 and MH 00219).

RELATIONSHIP BETWEEN FUNCTIONAL ACTIVITY AND GLUCOSE UTILIZATION IN THE RAT SUPERIOR CERVICAL GANGLION IN VIVO. P. Yarowsky, J. Jehle*, D. H. Ingvar*, and L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

The rat superior cervical ganglion (SCG) was studied as a model system for correlating in vivo glucose utilization, as measured by the deoxyglucose method (SOKOLOFF et al. J.Neurochem. 28:897, 1977), with electrical activity. The SCG is particularly suitable for such a study. One can modulate and monitor both the preganglionic input as well as the output of the postsynaptic neurons. Three different experimental conditions have been studied: normal controls, unilateral decentralization, and electrical stimulation. In normal conscious rats, mean (± S.E.M.) glucose utilization of the SCG in vivo was 35 (± 1) µmoles/100 g/min (n=20) with no significant side-to-side difference. This value is higher than the in vitro glucose utilization at 37° of the SCG, 23 µmoles/100 g/min, reported by Horowitz & Larrabee (J. Neurochem. 9:407, 1962). Glucose consumption was not uniform throughout the SCG. In the rostral portion of the SCG the rate of glucose utilization was 3 times higher than the rate in the rest of the ganglion. This region of high metabolic activity corresponds to the anatomical distribution (demonstrated by the horseradish peroxidase technique) of postganglionic neurons which give rise to the axons of the internal carotid nerve (Bowers & Zigmond, J.Comp.Neurol. 288:227, 1979). In unilateral decentralized animals, the left cervical sympathetic trunk (CST) was severed at least 1 cm from the SCG, and a piece of the nerve was removed 7-9 days prior to the measurement of glucose utilization. Following the decentralization, the animals developed both miosis and ptosis unilaterally. The mean (± S.E.M.) glucose utilization of the decentralized SCG was reduced to 22 (± 2) µmoles/100 g/min (n=8), and the region of high activity was obliterated. Glucose utilization in the contralateral control SCG was the same as that found in the normal animals. Experiments in which the CST was electrically stimulated were carried out under urethane (1 g/kg) anesthesia. The CST was placed on platinum electrodes and stimulated via a stimulus isolation unit. Stimulation was initiated 5 min prior to the onset of measurement of glucose utilization. In all cases stimulation caused ipsilateral miosis and widening of the palpebral fissure. With stimulation frequencies of 5, 10, and 15 Hz, there was an apparent frequency-dependent increase in the mean rate of glucose utilization of the SCG above control levels under urethane anesthesia. The region of high utilization was still present and enhanced. These studies suggest that the glucose consumption of the SCG is modulated by functional and/or electrical activity.
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1431 NUCLEI PARAGIGANTOCELLULARIS LATERALIS IN THE RAT: ANALYSIS WITH GOLGI IMPRINTS. Joseph A. Andreizik. Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

The nucleus paragigantocellularis lateralis (PGCL) lies lateral to the medullary pyramid and extends caudally from the trapezoid body through the rostral third of the inferior olive nuclear mass. At least eight neuronal types of the PGCL can be distinguished in Nissl preparations, and some of these are distributed preferentially within the nucleus. On the basis of cytoarchitecture and the distribution of afferent fibers, the PGCL may be divided into rostral and caudal subdivisions (Andreizik and Chan-Palay, Anat. Rec. 187:524, 1977).

Brains of adult Sprague-Dawley rats were impregnated with the rapid Golgi, Golgi-Cox, or several variants of the Golgi-Kopsch technique. The brains were sectioned in coronal, horizontal, or sagittal planes for examination.

At caudal levels, the dendrites of most PGCL neurons are oriented in the coronal plane with the longest dendrites extending in the mediolateral direction. Examinations of sections cut in horizontal or sagittal planes reveal a few categories of neurons whose arborizations are nearly equal in transverse and longitudinal extent. One type of arborization is essentially planar—dendrites abut directly opposite or at right angles to one another, often resulting in a cruciate appearance. Another type exhibits a dendritic tree forming an apex from an arbor in the pyramidal-shaped perikaryon. Dendrites of some other neurons form a sphere with the perikaryon at the center. At more rostral levels, PGCL neurons are situated medial to the facial motor nucleus. A striking feature in this area is that dendrites of PGCL neurons follow the contours but do not penetrate the borders of the facial nucleus.

Most PGCL neurons have relatively unbranched dendrites and a smooth surface. Some, however, exhibit spines on the perikaryon and dendrites, but these are rare. Spines usually are simple in form, being either sessile or pedunculated. Dendritic bundles are found in the PGCL and are formed by 5-7 dendrites each from a different neuron. The bundles are surrounded by fascicles of passing fibers and are more easily visualized in sections cut in coronal or horizontal planes. The various forms of PGCL neurons probably reflect the number and types of afferents which contact them and ultimately influence their activity.

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An ultra-rapid freeze apparatus has been designed to study vesicle dynamics at peripheral noradrenergic terminal axons. (1) It utilizes extremely thin strips of blood vessels and vas deferens. The apparatus consists in linear sequence of (1) a warmed superfusion chamber for stimuli­
ing and recording, (2) two closely opposed copper blocks precooled to liquid nitrogen temperature, (3) a similarly pre-cooled tissue vise specially constructed to mount in a Denton freeze fracture apparatus (J. Neuhoff et al., 1979), and (4) a taught spring terminating as a belt, tissue and carrier combination is drawn toward the copper freezing blocks where the tissue carrier stops. The belt and tissue continue to freeze, freezing blocks leading to the vice, where the tissue comes to rest some 2-3 sec later.

A layer of tissue was brought into the ultra-rapid freeze apparatus and was separated from the freezing blocks by an air gap of less than 1 sec. This gap was observed to be of critical importance in allowing the tissue to achieve the deep subzero temperatures necessary for the freezing of nerve terminals. This, we believe, is the first report of myelinated dendrites in the olfactory bulb of rodents and of myelinated neurons in the mouse olfactory bulb. Observations were made using the Golgi technique. The myelinated neurons appear to have a laminar distribution with layers of control and experimental mice of olfactory nerve fibers, but mitral cell, internal plexiform, and granule cell layers were also present. Mice were 12-14 weeks old. Olfactory bulbs were cut into 1-2 mm blocks, osmicated, and embedded in Spurr's medium. Sections were stained with UA and IB. Many small dendrites and perikarya of the olfactory bulb were myelinated, and no difference was observed in the relative frequency of these myelinated structures in control and experimental mice. Large dendrites with 6-10 lamellae of myelin were observed in the periglomerular region of the glomerular layer and external plexiform layer. Identification of the myelinated dendrites was based on the large diameter and cytological details of the myelinated dendrites. Myelinated dendrites contained: rough endoplasmic reticulum, free ribosomes, mitochondria, smooth endoplasmic reticulum and microtubules. On one myelinated dendrite cut in longitudinal section, synaptic contacts were present amongst a portion of the dendrite devoid of the myelin sheath. A thinner layer of myelin (2-5 lamellae) was observed to surround partially the sparse myelinated neurons present in the periglomerular region and the external plexiform layer. From a comparison of these results with the descriptions of normal neurons in the olfactory bulb by Pinch and Powell (1977), these myelinated neurons appear to be small tufted cells or axon collaterals. They contained: moderate amount of rough endoplasmic reticulum, free ribosomes, mitochondria, stacks of Golgi, slightly indented nuclei, and frequently a well-developed myelin sheath. The origin of the myelin sheaths could not be determined for all the myelinated profiles. Oligodendrocytes may contribute myelin lamellae to the myelinated dendrites and perikarya, but this was not observed. However, in a few instances, myelin lamellae surrounding small myelinated axons were observed to leave the axon and contribute myelin lamellae to the myelinated dendrites or perikarya.

Supported by: NS 12440, MH 14277, and Alfred P. Sloan Fnd.
1435 FINE STRUCTURAL ANALYSIS OF THE MOTOR TRIGEMINAL NUCLEUS OF THE RAT. John F. Cast4 and Robert V. Moore (Spon: J.C. Bipe). Department of Neuroscience, The UCSD, La Jolla, California 92037.

Recent histofluorescence examination of the monoamine innervation of various brainstem nuclei of the rat (Levit and Moore, 1979) have demonstrated that the motor trigeminal nucleus receives a heavy noradrenergic innervation from lateral tegmental cell groups. The present investigation was therefore undertaken to examine the ultrastructural organization of this nucleus with a basis for further fine structural analysis of the pattern of catecholaminergic innervation of this area. Adult male rats were anesthetized with sodium pentobarbital and subjected to cardiac perfusion fixation with glutathione-parvaldehyde solutions. The motor trigeminal nuclei were then dissected from the brainstem and prepared for electron microscopic examination using conventional procedures. Large (40-60 μm) motor trigeminal neurons were a prominent feature among the numerous large dendritic trunks and are characteristic of the neuropil in this region. The cytoplasmic constituents of these cells were typical of those generally described in axons throughout the neuraxis. In particular, of primary interest in the present study was the large number of axonomatic contacts which were routinely observed on the perikarya of all cells examined. In some instances, as much as 94% of the cell soma was covered by axon terminals. Such terminals could be subdivided into categories on the basis of axoplasmic density and vesicle size. The presence of either clear or dense axoplasm within the endings provided an easily recognized morphological criterion for subdividing axonomatic terminals into two distinct populations. In addition, analysis of vesicle morphology within endings indicated that both clear and dense terminals could be further subdivided on the basis of their vesicular content. In each case, endings were observed which contained either exclusively lucent spherical vesicles of about 100-150 μm) or microvesicles (0.06-0.1 μm) and flattened (0.06 μm) vesicles. Thus, four morphologically distinct terminal types have been identified in synaptic contact with the soma of the MTN neurons. In addition, analysis of the neuropil of the MTN revealed that the numerous dendritic profiles characteristically present in this area were also contacted by the same types of terminals observed on the soma. Whether or not the distinct morphological differences in axonomatic and axodendritic endings reflect differences in neurotransmitter content cannot be determined at the present time. However, studies are presently underway to determine if the dense noradrenergic innervation of this nucleus can be correlated with one or more of the demonstrated terminal morphologies. Supported by USPHS Grant NS 12080.

1436 APPLICATION OF HIGH MAGNESIUM CONCENTRATION PERSEPTION-FIXATION TO RAT VISUAL CORTEX. Fen-Chi Chang, Mary Kay Floeter* and William T. Greenough, Dept. Psychol, and Neural & Behav. Biol. Prog., Univ. of Ill., Champaign, Ill. 61820.

Chang (J. Neurocytol., 1974:3:133) suggested that artificially low cortical densities might result from normal sodium glutaeraldehyde fixation procedures due to the activation of transmitter release by glutaraldehyde. He used a high Mg2+ concentration (110 mM) to reduce the rate of transmitter release. We report here that Mg2+ concentration of cervical ganglion to block synaptic transmission and found dense packing of vesicles in about 25% of synapses. McKinlay & Upholt (J. Urol., 1974:111:101) suggested that glutaraldehyde can increase the frequency of miniature EPSPs at locust neuromuscular junction, and that addition of Mg2+ attenuates this effect. We have performed experiments to determine concentration during fixation and found a very dense vesicle packing in synaptic terminals. We have applied to rats' mother rat visual cortex. Three concentrations of Mg2+ (MgCl2) were used - 110, 160 and 210 mM. Adult rats were anesthetized with pentobarbital and perfused through the heart first with 0.9% NaCl, then with a high Mg2+ solution (besides the MgCl2). 500 ml of the same vehicle with glutaraldehyde added to a final concentration of 3%. After fixation in the same fixative for 1 hour, 1 mm tissue blocks were dissected and post-fixed for 1 hour with 1% OsO4 in sodium phosphate buffer. Tissue was embedded in Epon, sectioned in silver to a thickness range of about 50-70 μm and stained with uranyl acetate and lead citrate.

Preliminary results using this technique indicate that 1) High Mg2+ perfusion does appear to result in fixation in which a high vesicle packing density of synaptic vesicles can be observed. These terminals. There is a tendency in some, but not all cases, for vesicles to be aggregated in the center of the terminal (away from the synaptic cleft), while outside the synaptic cleft there are absolutely greater numbers of vesicles per terminal are present with Mg2+ perfusion remains unclear. Quantitative studies are in progress). 2) 110 mM Mg2+ was sufficient to result in increased vesicular packing density. 3) With higher concentrations there is some indications of increased extracellular space and/or shifts of vesicles in the cleft. Whether or not these differences are due to increases in vesicles, but the aggregation of vesicles seems not to be accounted for in terms of shrinkage of presynaptic terminals. Work is in progress to evaluate the effects of a wider range of Mg2+ concentrations as well as chelating agents and other divalent ions. Supported by NSF BMS 7722660.


The intermediolateral nucleus (IMLN) of the thoracic cord in the rat was studied by using fluorescent and electron microscopic techniques. As visualized with the fluorescence microscope, the IMLN somata primarily are surrounded by dense axonal varicosities and terminal boutons. At regular intervals these somata aggregate to form "islands". These aggregations are bridged by fluorescent perikaryal fibers which presumably denervate these processes. At an early stage (2½ days) after animals were treated with either 6-hydroxydopamine (6-ODHA) or 5,7-dihydroxytryptamine (5,7-DHT) denervation procedures, the IMLN was studied with TEM. In these sections, the perikarya were usually interrupted or less dense. Special interest was paid to perikarya in the electron microscope, the IMLN somata often have a fusiform shape, a high nucleus to cytoplasm ratio, an extremely light nuclear chromatin, and a paucity of cytoplasmic organelles. Dendritic elements are long and interwoven among unmyelinated axonal bundles in a parallel and longitudinal fashion. This longitudinal arrangement of axonal and dendriticplexuses and terminals demarcates the IMLN zone and is a characteristic feature of this nucleus.

The synaptic arrangement in the neaural is mainly axo-dendritic which could consist of one axonal terminal contacting several dendrites or vice versa. There are both clear and dense terminals, and axo-axon, but contacts are comparatively rare. Sometimes these contacts form serial synapses. In general, approximately 45% of the synaptic terminals could be subdivided on the basis of their vesicular content. In each case, endings were observed which contained either exclusively lucent spherical vesicles of about 100-150 μm) or mixed populations of spherical and flattened (0.06-0.1 μm) vesicles. Thus, four morphologically distinct terminal types have been identified in synaptic contact with the soma of the MTN neurons. In addition, analysis of the neuropil of the MTN revealed that the numerous dendritic profiles characteristically present in this area were also contacted by the same types of terminals observed on the soma. Whether or not the distinct morphological differences in axonomatic and axodendritic endings reflect differences in neurotransmitter content cannot be determined at the present time. However, studies are presently underway to determine if the dense noradrenergic innervation of this nucleus can be correlated with one or more of the demonstrated terminal morphologies. Supported by USPHS Grant NS 12080.


The third ventricle of normal ewe and ram lambs (four - six months of age) was examined by scanning (SEM) and transmission electron microscopy (TEM). SEM of the ventrolateral floor (VF) revealed few nerve fibers. The distribution of intraventricular fibers was studied with SEM on the third ventricle of an immature ewe. The ram lamb VF had a more patchy appearance due to fewer fiber features on some cells. Distinct fibers were not observed in the VF of the 6- to 8-week-old ram. The developing VF of a 6-week-old ram had small clear vesicles, thickly ciliated. Underlying ependymal surfaces could not be observed except in places where the cilia were parted, revealing a single cell studed with cilia and overlying a thin layer of clear vesicles. Whether or not these new structures could be interpreted as nerve fibers remains unclear. In these more exposed areas, SEM images which might be interpreted as nerve fibers in the DF were rare. Yet when the same region was studied in the 10-week-old ram, more nerve fibers were revealed along the ventricular surface of the DF. TEM revealed large clear vesicles in the ventricular surface of the DF. Small caliber unmyelinated nerve fibers contained small clear vesicles, occasional small dense core vesicles, neurotubules and mitochondria, and were interpreted as axons seen in section. Occasional attachment plaques between axons and the ependyma, and microvilli partially wrapped around some nerve fibers were noted. Single fibers as well as groups of fibers suggesting plexus-type organization were present. Possible myelinated fibers were rare. A noteworthy, though rare finding under the DF cilia were large pear-shaped dense core vesicles which were routinely observed on the perikarya of all cells examined. In some instances, as much as 94% of the cell soma was covered by axon terminals. Such terminals could be subdivided into categories on the basis of axoplasmic density and vesicle size. The presence of either clear or dense axoplasm within the endings provided an easily recognized morphological criterion for subdividing axonomatic terminals into two distinct populations. In addition, analysis of vesicle morphology within endings indicated that both clear and dense terminals could be further subdivided on the basis of their vesicular content. In each case, endings were observed which contained either exclusively lucent spherical vesicles of about 100-150 μm) or mixed populations of spherical and flattened (0.06-0.1 μm) vesicles. Thus, four morphologically distinct terminal types have been identified in synaptic contact with the soma of the MTN neurons. In addition, analysis of the neuropil of the MTN revealed that the numerous dendritic profiles characteristically present in this area were also contacted by the same types of terminals observed on the soma. Whether or not the distinct morphological differences in axonomatic and axodendritic endings reflect differences in neurotransmitter content cannot be determined at the present time. However, studies are presently underway to determine if the dense noradrenergic innervation of this nucleus can be correlated with one or more of the demonstrated terminal morphologies. Supported by USPHS Grant NS 12080.
The extraordinary accumulation of vesicles in this nerve, it is highly refractile and easily distinguished from other nerves by phase contrast optics. Cobalt backfills have localized small patches of the substrate with Schwann cells from the SN-DRG cultures were transferred to neuritic regions of the N-DRG cultures. Such recombiant groups included: 1) dystrophic Schwann cells onto normal neurites, 2) normal Schwann cells onto dystrophic neurites and 3) dystrophic Schwann cells onto dystrophic neurites. By five weeks after transplantation, Schwann cells had proliferated and migrated to occupy a substantial region of the neuritic outgrowth. At this time, the cultures were fixed and examined by electron microscopy. Schwann cells derived from dystrophic mice had a characteristic patchy basal lamina when related to either normal or dystrophic neurites. Schwann cells derived from normal mice produced a thick, uniform basal lamina when cultured on dystrophic mouse neurites. The basal lamina abnormality in tissue culture is therefore an expression of an abnormality of the dystrophic Schwann cell rather than of the neurite.

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SUPAEPIPENDYAL AND EPIPLEXUS CELLS IN BODENTS. Perry Cohm (SPON: Ruth Bleier). Dept. Neurophysiol., Univ. Wis., Madison, WI 53706

Abundant monocyte and macrophage-like supaeipendymal (SE) and epiplexus cells were observed with transmission and scanning electron microscopy in neonatal rats, mice and hamsters. In white Boltzman rats, which were most extensively studied, their numbers are elevated from 3-20 days of age. Some display micro-processes, ridges and ruffled and spreading lamellipodia, others have smoother surfaces and lengthy, branching pseudopods and still others are rounded. Their ultrastructural characteristics include an extensive and varied group of lysosome-like organelles, prominent Golgi zones, a nucleus possessing clumped, margminated chromatin and often a number of Jucent 500-2000 nm vacuoles. Cilia are often deeply invaginated into SE cells. Occasional cells resemble lymphocytes and epiplexus cells.

The macrophage-like SE cells are usually found in the relatively unciliated areas of the ependymal walls. The remaining descriptions are of the well studied third ventricle. In the fetus and the newborn SE cells can be seen throughout the ventricle, which at this time is mostly unciliated. In sucking rodents more than a few days old, the relatively unciliated area is restricted to an area roughly overlying the hypothalamic ventromedial, infundibular and preemisillary nuclei and harbors about 75-100 SE cells per side. Most are found rostrally of caudally in this area. In adult rats the few SE cells (10-20 per side) congregate near the border between the ciliated and unciliated ependyma.

In sucking rats macrophage-like cells are also associated with capillaries that cross the ventricular lumen and with clusters of cells on the floor of the ventricle. The ultrastructure of the clustered cells includes a euchromatic nucleus, a prominent nucleolus, an active Golgi zone, a smooth surface and occasional synaptic endings on their surface. They are often embedded in a coat of fibers. As many as three separate clusters may be present.

The ependymal cells overlying the infundibular nucleus display many rounded excrescences (commonly known as blebs) in sucking female rats over 2 days old. Some male rats 3 to 6 days of age also have blebs. In adult rats preliminary investigation of the estrus cycle revealed little predictable variation. SE cells are not observed particularly over ependyma displaying blebs, though they are usually located nearby.


This communication describes the morphology of a neurosecretory tract in the cricket having exceptional properties for experimental study. A small (17u) nerve emerges from the anterior end of the last abdominal ganglion in the cricket, Acheta domesticus. This nerve courses anteriorly between paired connectives and inserts into the next to last ganglion between connectives. Two-thirds of the way along this tract two branches emerge and extend laterally. These branches ramify just short of the cuticle and may function in spiracle opening.

Electron microscopy shows that this nerve contains approximately 18 axons most of which are densely filled with neurosecretory vesicles. Because of the great accumulation of vesicles in this nerve, it is easily distinguished from other nerves by contrast optics. Cobalt backfills have localized 21 of the cell bodies contributing axons to this tract from the last abdominal ganglion. These occur in three groups of three soma each lying along the anterior dorsal and anterior ventral mid-line. Two other cell bodies are laterally disposed. One group of three cells occurs at the point of departure of the connectives from the dorsal ganglion and should be easily sampled by electrode and E.M. sectioning techniques. Cobalt fills have also identified four cells in the next to last ganglion which contribute posteriorly coursing axons to this tract. The extraordinary accumulation of neurosecretory vesicles in this nerve (not just its ending) and the ability to easily locate several of the neurosecretory cell bodies makes this system especially amenable to experimental study.
NEUROCYTOLOGY


Previously, we have found a massive and transient increase in the concentration of the "smooth-walled coated vesicles (DVCV)" on the surface membranes of the cebellar glomerulus during late postnatal development in the mouse. In an attempt to determine whether this was specific to the cebellar glomerulus or a more general phenomenon during brain development, we have examined another brain region. Since similar structures were reported in adult mitral cell bodies of the olfactory bulb we examined these cells. The purpose of this study was to describe the distribution and frequency of occurrence of DVCV's in the mitral cell bodies during postnatal development. Mitral cell bodies were perfused through the left ventricle with dilute Karnovsky's fixative at 1, 5, 10, 16, 20, 26, 37 and 70 days of age. The olfactory bulbs were removed and prepared for electron microscopy.

We frequently observed DVCV's in random electron micrographs of mitral cell bodies. Most of these DVCV's consist of a coated evagination of the plasmalemma of the mitral cell body and an inner evagination of the plasmalemma of either an adjacent granule cell axon or mitral cell body. Also, coated invaginations of the plasmalemma of the granule cell axon were observed with inner evaginations of the plasmalemma of the adjacent mitral cell body. In addition, DVCV's were observed free in the cytoplasm of the mitral cell bodies and granule cell axons. A morphometric analysis revealed that the highest numbers of DVCV's were found at 37 days (0.035 DVCV's per µm of mitral cell cytoplasm), some 23 fold more than at 1 day and 5 fold more than 70 days of age. It is determined whether there was a relationship between the occurrence of DVCV's and synaptogenesis, the number of synaptic terminals per µm of mitral cell body, or the content of synaptic terminals on the mitral cell bodies reached adult levels by the 20th day. The higher frequency of occurrence of DVCV's at 20 and 37 days of age follows synaptogenesis, as we observed in the cerebellum. Thus, DVCV's are present in highest numbers during late postnatal development in at least two brain regions. It is possible that the formation of DVCV's may provide a mechanism for removal of specific surface constituents present on immature membranes.


THE MONKEY CLAUSTRUM: AN ELECTRON MICROSCOPIC ANALYSIS

Lawrence R. Edelstein and Frank J. Denaro, Dept. of Anatomy, SUNY at Stony Brook, Stony Brook, NY 11794

While the claustrum has been studied for several years on both a light- and electron-microscopic level, there is a paucity of research in terms of the monkey claustrum. Of late, the use of HRP has aided investigators in the study of the monkey claustrum. (Riche & Lanoir, JCN 177: 435, 1978). However, to our knowledge, the only published EM study done prior to the use of the HRP was carried out by Norita (Acta Anat. Nippon. 49: 47, 1974), who gave a brief report on the claustrum of Macaca irus.

While under Nembutal anesthesia, the subject was initially perfused with physiological saline at body temperature, followed by a second perfusion with fresh 1.5% osmium tetroxide. In thin sections (60nm) examined at 80kv, and in thick sections (.25um) at 1 Mev (at the HVEM facility supported by NIH Career award AG00016 and NIH grant AG00001). The predominant cell type in the sections studied was slightly elongated (-10um), displaying a large invaginated nucleus (containing chromatin clumps) with a thin peripheral rim. While some processes, which are postsynaptic to large terminals, are primary afferent terminals distributed along the cerebral cortex and in normal EM-perfused macaque area 17 and hippocampus.

The purpose of the present study was to examine, in rapid Golgi preparations, the form of astrocytes in human temporal neocortex and hippocampus. Of interest, since our knowledge about the stratification of the claustrum is quite limited, the Golgi findings were similar in all 6 blocks. The major findings were also confirmed in a separate surgical block from human frontal cortex and in normal EM-perfused macaque cerebellum. For these and other reasons, such cells are considered to be the velate-type astrocytes of Palay & Chan-ya. While both R and S primary processes occasionally display a pyknotic nucleus, they are quite densely studded with excrescences and terminate bluntly. A small number of them, however, which frequently extend somewhat beyond the typical primary process length, give rise to distal filaments forming a thin layer that are somewhat larger than at 1 day and 5 fold more than at 20 and 37 days of age. The higher frequency of occurrence of DVCV's follows synaptogenesis, as we observed in the cerebellum. Thus, DVCV's are present in highest numbers during late postnatal development in at least two brain regions. It is possible that the formation of DVCV's may provide a mechanism for removal of specific surface constituents present on immature membranes. (Supported by NIH Grants NS 10657 and 11325.)

1446 AN EM AND HVEM STUDY OF NEURONS AND SYNAPSES IN THE FELINE DORSAL COLUMN NUCLEI. Leland C. Ellis, Jr. and Aldo Rustioni, Depts. of Anatomy and physiology, UNC, Chapel Hill, N.C. 27514

With the human newborn, the thalamic relay neurons (TRN) labelled with HRP are typically round, average approx. 25um in diameter, and have a nucleus that is often highly indented or lobulated. While the ALPs are unbranched, lack excrescences, and terminate distally in endfeet about 6-10µm in diameter and of highly irregular form. In all cases, ALP knobs and R and S endfeet are in contact with vascular perikaryon containing poorly organized mitochondria and a fair number of round and elongated ribosomes and a fair number of round and elongated mitochondria. Axo-axonic synapses were noted. Most of these ALPs are axo-axonic, axo-dendritic and axo-axonic synapses were observed, with a predominance of the axo-dendritic variety. Of interest, since our knowledge about the stratification of the claustrum is quite limited, the Golgi findings were similar in all 6 blocks. The major findings were also confirmed in a separate surgical block from human frontal cortex and in normal EM-perfused macaque cerebellum. For these and other reasons, such cells are considered to be the velate-type astrocytes of Palay & Chan-ya. While both R and S primary processes occasionally display a pyknotic nucleus, they are quite densely studded with excrescences and terminate bluntly. A small number of them, however, which frequently extend somewhat beyond the typical primary process length, give rise to distal filaments about 6-10um in diameter and of highly irregular form. In addition, a small number of primary R processes continue as long (up to ~70µm), radially oriented axo-like processes (ALPs). ALPs are unbranched, lack excrescences, and terminate distally in axo-axonic synapses. A small number of S processes, of somewhat greater length, bear endfeet similar to those of cell primary processes. ALPs are located in the superficial territories (~100µm in diameter). Primary processes branch frequently and form thick perisomatic tangles. The processes are quite densely studded with small spine-like and irregular excrescences and usually taper to pointed tips. Most of these ALPs are axo-axonic, axo-dendritic and axo-axonic synapses were observed, with a predominance of the axo-dendritic variety. Most of these ALPs are axo-axonic, axo-dendritic and axo-axonic synapses were observed, with a predominance of the axo-dendritic variety. Most of these ALPs are axo-axonic, axo-dendritic and axo-axonic synapses were observed, with a predominance of the axo-dendritic variety. Most of these ALPs are axo-axonic, axo-dendritic and axo-axonic synapses were observed, with a predominance of the axo-dendritic variety.

The purpose of this study was to observe the cytology of the axon reaction of different cranial neurons in the adult hamster. In one group of animals the right facial and hypoglossal nerves were severed, while another group of animals underwent a right vagotomy. The left nerves remained intact to serve as a control. Animals were allowed to survive 5, 30 and 60 days postoperative (dpo). Following fixation brain stems were double-stained for the neuronal cytoskeleton and lamina on light microscopic sections. Tim F. Kowalski, Earl R. Anson, and Gary E. Korte*. Dept. Biobehavioral Sci., Univ. of Connecticut, Storrs, CT 06268

We recently identified three types of synaptic boutons in cat superior vestibular nucleus, based on their vesicle content and synaptic sites. Unilateral destruction of the vestibular ganglion resulted in loss of one type ipsilateral (lesioned side) but not contralateral (control side). In addition, we measured synaptic vesicles and found that the three putative types differed. We have since analyzed the data on synaptic vesicles with two widely available statistical 'package' programs which perform classification of cases from numerical data: DISCRIMINANT, Statistical Package for the Social Sciences; and HSPMN, cluster analysis, Biomedical Computer Programs.

The mean size and some other statistics on the vesicles in each bouton were entered into the discriminant program. In the 'training' phase, the program compared the three groups, as defined by us; in the 'classification' phase, each ending was reclassified solely from the vesicle data and without reference to our subjective label.

Agreement between our classification and that of the discriminant program was better than 95%, when trained on the control side data only, the program repeated to 90% agreement our tabulation of the endings on the lesioned side and confirmed the preferential loss of the putative primary afferent type.

The cluster program associated the endings into a hierarchy of progressively larger and more heterogeneous groups; the pattern agreed with our subjective classification and indicated the presence of endings which we had not predicted visually.

The discriminant analysis described here required the prior imposition of subjective labels to each ending, an undesirable feature. Subjective labeling may be less necessary when techniques such as axonal transport, intracellular injections or cytochemistry are employed.

Our experience and that of these two programs, which are examples of statistical pattern recognition analysis, can classify synapses as well as human observers, when given good data. Furthermore, even in our apparently simple paradigm, one program detected patterns unnoticed visually. The approach of morphometry and statistical pattern recognition analysis may be generally useful in the analysis of synaptic connections by electron microscopy. Supported by NIH: NS09994; I P24NS05553; T32GM07219-01A1 HCL.

A REVERSIBLE DEPLETION OF SYNAPTIC VESICLES INDUCED BY A SINGLE-GENE MUTATION OF DROSOPHILA MELANOGASTER. Kazuo Ikeda and Kogaku Salt6. Div. of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010, USA.

It has been shown that a reversible, temperature-dependent block of neurotransmitter transmission occurs in the single-region mutant, shibire51 (sh51). The neuronal junction of the dorsal longitudinal motor flight muscle was observed by electronmicroscopy. sh51 and Oregon-R, were raised at a constant temperature (17° C). Four-day old adult flies were dissected in a phosphate buffer and fixed in a paraformaldehyde-gluceraldehyde mixture. The nervous system was processed for electron microscopy after fixation were then done at 19° C. The structure of the sh51 synapse showed almost complete depletions of vesicles and in addition, many cisterna-like structures appeared. The Oregon-R synapse remained the same as at 17° C. In order to see the reversibility of the temperature effect, sh51 was exposed to 29-30° C for 20 min, prior to dissection. The dissection and fixation were then done at 17° C. The structure of the sh51 synapse was returned to normal, i.e., the cisterne-like structure disappeared and the vesicles reappeared. Since high temperature (29-29° C) causes motor output to this muscle in sh51, the depletions may have to be caused by temperature-induced synaptic input. To determine this, the nerve innervating this muscle was cut before exposure to high temperature. The results were the same in those cases without the nerve intact. Thus, the depletion is a direct result of the mutation on the presynaptic terminal, rather than the result of postsynaptic activity. Our previous physiological finding that the neuromuscular transmission of sh51 is reversibly blocked by high temperature is consistent with the morphological findings. We have since analyzed the data on synaptic vesicles with two programs, which are inappropriate for permanent light microscopic preparations. We have developed a staining technique for epithelial synapses which is highly specific and sensitive, permanent, relatively inexpensive and is suitable for light or electronmicroscopy (EM). The basement membrane (BM) from CNS tissue of inogenic female albino Wistar rats was isolated by the technique of Meschan (1975). Using this BM preparation as an antigenic source, we developed a hyperimmune serum. This serum was exhaustively absorbed on rat CNS BM antigen. Using this BM preparation as an antigenic source, we developed a hyperimmune serum. This serum was exhaustively absorbed on rat CNS BM antigen. Using this BM preparation as an antigenic source, we developed a hyperimmune serum. This serum was exhaustively absorbed on rat CNS BM antigen.

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1451 ULTRASTRUCTURE OF THE SONA OF AN IDENTIFIED DOPAMINE-CONTAINING NEURON OF THE SPINY LOBSTER CNS. Pinky Drosten Kushner and Eric Schachtman, Dept. of Biology, U. of Oregon, Eugene OR 97405. Each commissural ganglion of the spiny lobster contains a large (150 µm) neuron, readily identified by its characteristic somal position and many neurite projection within the ganglion. This cell exhibits catecholamine histofluorescence (Kushner and Maynard, Br. Res.129 13, 1977), synthesizes dopamine from labeled tyrosine (Barrett, Kushner and Hooper, Br. Res.161 99, 1979), and contains measurable amounts of dopamine (0.51 nmoles) (Kushner and Omo, Neurosci.Abs.d 610, 1978). We have examined its ultrastructure seeking possible unique morphological features.

The cell is characterized by dense cytoplasm with closely packed rough and smooth endoplasmic reticulum, in random array. There are numerous Golgi (~8 Golgi/10 µm), in close proximity to which are vesicles of varying sizes and densities, with many coated vesicles on the concave side. Multivesicular bodies are common with inclusions of small irregular vesicles of varying densities. Although the fixation we used was designed to emphasize densely staining vesicles associated with biogenic monoamines (Wood's fix as modified by Friend, Cell Tiss. Res. 175 369, 1976), we see few typical dense-cored vesicles, either Golgi associated or cytoplasmic.

paraaxial swirls of microtubules concentric to the nucleus funnel into the large primary neurite. The nucleus is small (25 µm) relative to the size of the soma and possesses a smooth unindented nuclear membrane.

This somal architecture is very different from that of the identified mollusc dopamine neuron (Anodontia: Zs.-Nagy in Neurobiology of Invertebrates, ed. Salamki, 1968; Planoebia: Berry in Biochemistry of Characterized Neurons, ed. Osborne, 1978) where granular vesicles are abundant. Likewise the octopamine neurons of locust have an ultrastructure (Hoylcy, J. Neuropol., in press) which is more similar to the mollusc dopamine neuron than to this lobster dopamine neuron. The paucity of densely precipitating vesicles is a feature shared with the presynaptic dopamine neuronal somata of the vertebrate substantia nigra (Hokfelt and Ungerstedt, Br. Res. 60 269, 1973).

1453 OBSERVATIONS ON THE FINE STRUCTURE AND CONNECTIONS OF THE AREA SUBPOSTREMA OF THE CAT. R. A. Leslie and D. G. Gwyn. Dept. Anat., Sch. Med., Dalhousie Univ., Halifax, N.S., Canada B3H 4H7. The area subpostrema (ASP), corresponding to the parvocellular and parvocellular parts of the nucleus of the solitary tract, has been examined with the electron microscope. In some cases the nodose ganglion was removed unilaterally several days prior to microscopical study. The nervous complement of the ASP was seen to consist of a population of a single type of small cell bodies, about 10 µm in diameter. Nuclear membranes were very deeply indented and were linked to each other with gap junctions. The associated glial cell bodies were usually closely apposed to the neuronal somata.

Membrane bundles of the, unmyelinated axons coursed through the ASP usually in a rostro-caudal direction. Typical axo-dendritic and axo-somatic asymmetric synapses were seen to contain either round, clear vesicles or pleomorphic vesicles. Some round vesicle-containing terminals also contained a few large (80 nm diameter) dense-cored vesicles. Dendo-dendritic and dendro-somatic synapses were occasionally seen and contained only pleomorphic vesicles. Very rarely an axo-axonal synapse was seen associated with a terminal containing round, clear vesicles. Degenerating terminals of vagal origin contained round, clear vesicles together with occasional large dense-cored vesicles. These terminals seemed to make up only a small percentage of the axo-dendritic synapses of the ASP and occurred singly throughout the substance of the area. Some of these were of gastriac origin since the ASP has been recently shown to receiveafferent fibres from the wall of the stomach (Owen, Leslie and Hopkins, 1979).

1454 POTASSIUM FERRICYANIDE: AN IMPROVEMENT IN NEURAL FIXATION. Lauren A. Langford and Richard E. Coggeshall. Departments of Anatomy and of Physiology and Biophysics, The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas, 77550.

Changes in nuclear structure, a primary neuronal response to axotomy indicating alteration in RNA and protein metabolism, have been especially well characterized in adult hamster facial neurons. An intranuclear body (INB), composed of ribonucleoprotein, is produced in the nucleus of most of these neurons. The formation of the INB, the final step in nuclear cytomorphology, occurs well after adult normal size and Nissl body configuration are present. Amputation of the facial nerve significantly delays the appearance of the INB in the adult and prevents its normal formation in development. This study examined the long term effect of injury to developing neurons on their nuclear morphology as seen with the light microscope.

INB formation was examined after either ligature/axotomy or crush performed at 15 days postnatal age before the INB had formed, at 20 days when the INB was just beginning to form, and at 25 days when the mature nucleus was present. Animals were sacrificed at 4, 10, 15, 30, 50, 100 and 200 days after axotomy.

For all operative areas, crush injury resulted in complete INB recovery by 50 days. By 200 days INB return was still at control levels. With regeneration, the state of nuclear maturation at the time of operation did not seem to be a decisive factor in eventual INB return after crush injury.

Although both types of injury extended the normal timetable for nuclear maturation by 15 days postnatal age drastically delayed the formation of the INB. Even by postoperative day 200 the number of INBs were significantly depressed on the injured side. Thus, 1)ig/axotomy, and the concomitant lack of reconnection, affected the long term capability of the youngest neurons to recover. This appeared dependent on the stage of nuclear maturation at the time of injury, 2)ig/axotomy at 20 and 24 days, the normal nuclear configuration was attained by postoperative day 100 and maintained at 200 days. Therefore, nuclear maturation at the time of injury, INB formation, was initiated, axotomy did not depress eventual full INB recovery.

In summary, injury was seen to result in long term alterations in nuclear configuration. Full INB recovery was dependent on the level of nuclear maturation at the time of injury only in the absence of regeneration. The many features changes correlate well with previous studies of the reactive metabolism of these neurons.

Galilas has introduced a set of six silver stains based on the principle of physical development (e.g., acta Neuruphath. 1970, 16: 33-53). In these methods a chemical development selectively enhances the argyrophilia of a given component of neural tissue. That component is then made visible by deposition of metallic silver through the "seeding" process of physical development, all components being equally treated. The application of physical development to silver impregnation of Nissl substance and to contrast enhancement of normal axons will be described.

In that physical development removes the limitation of tissue-bound 1457 the original cell population of the new optic nerve has been shown to respond to lesion over a time interval of the first 30 minutes after transection, involving a series of morphological and immunocytochemical events that may be related to the processes of regeneration. In these experiments, we have used the Fink-Schneider method for normal retina (Science 1969, 163: 685-902, note 31), in which high levels of background pigmentation impede visibility, particularly in tissue with short post-fixation time. After completion of the Fink-Schneider procedure, background pigmentation is removed from sections by a modified Farmer's Re- ducer. Silver is then deposited by physical development in greater amounts than originally present. The "bleach-intensity"


Mueller cells have been classified as modified retinal astrocytes (Polk 1941, Hughles and Coibins 1971) that span the thickness of the retina. These purported glial cells, recognized by classical morphology, have been the subject of a number of recent studies, attributing them with what have been traditionally considered neuronal functions. Some of these include storage and synthesis of neurotransmitters (Riepe and Norenburg, 1977), uptake and degradation of certain neurotransmitters (Sarthey and Lam, 1978) and electrophysiological activity, as demonstrated by the b-wave of the electroretinogram (Miller and Dowling, 1970).

The immunocytochemical technique of Sternberger, the per- oxidase-anti-peroxidase method, was employed to localize the GFA protein in formalin-fixed, paraffin-embedded 6 micron sections of the human eye. Rabbit antiserum to the GFA protein and seven human eyes obtained at autopsy were utilized. At the light microscopic level, the positive staining of the glial cells for the GFA protein clearly delineated the glial network of the retina. The GFA protein was present in the Mueller cells, throughout the retina, and represents the characteristic fine cytoplasmic projections. Since GFA protein has been localized specifically in astroglial elements (Eng and Rubinstein, J. Histochem. Cytochem. 27:513, 1979), this study confirms the classification of the Mueller cell as an astrocyte. With this technique, we were also able to partition the ora serrata into three distinct regions, representing the different stages of the Mueller cell morphology, on the basis of GFA protein staining.

Supported by the V.A., 1979.)


The glial cell population of the newborn retinal tract is far more complicated than that found in the adult retina. The most prominent area of staining reveals a laminated structure in the nerve fiber layer of the entire retina. We have observed that GFA protein was present in the Mueller cells, throughout the retina, and represents the characteristic fine cytoplasmic projections. Since GFA protein has been localized specifically in astroglial elements (Eng and Rubinstein, J. Histochem. Cytochem. 27:513, 1979), this study confirms the classification of the Mueller cell as an astrocyte. With this technique, we were also able to partition the ora serrata into three distinct regions, representing the different stages of the Mueller cell morphology, on the basis of GFA protein staining.

That component is then made visible by deposition of metallic silver through the "seeding" process of physical development, all components being equally treated. The application of physical development to silver impregnation of Nissl substance and to contrast enhancement of normal axons will be described.

Supported by NIH grant EY00269 to G. Schneider, and by a Whita- ker-Health Sciences Fund Fellowship to B. Merker.


The purpose of this study was to investigate the distribution and concentration of synaptic vesicles in nerve terminals of sympathetic ganglia following short periods of catechol stimulation. The method of rapid-freezing and freeze-substitution was utilized to determine reliably the distribution of synaptic vesicles in nerve terminals in the living state.

The ninth sympathetic ganglia of bullfrogs were used because most, if not all, preganglionic fibers enter through the trunk. Experiments utilized a specially designed stimulation - recording specimen carrier upon which ganglia were mounted, electrochemically stimulated and recorded, and finally lowered into a modified Van Hareveld apparatus for rapid freezing. All ganglia served as unstimulated controls and 5 were stimulated supramaximally, by way of the preganglionic trunks, at 20-25 Hz for 5 minutes. Rectangular pulses, 0.25-1.0 msec, were used. Postganglionic recordings were obtained from ganglial ram. After freezing, ganglia were processed by freeze-substitution in 2% OsO4 in acetone for 3 days at -80°C and then prepared conventionally for transmission electron microscopy. Several hundred preganglionic terminals near the surface of the ganglia were obtained for analysis.

Ultrastructural evaluation indicated synaptic vesicles in preganglionic nerve terminals had a more uniform distribution in rapidly frozen ganglia than those chemically fixed. Morpho- metric analysis of 40 control and 40 stimulated axosomatic synapses revealed that the concentration of clear-cored vesicles within 500Å of the plasma membrane of the active zone and in the entire terminal declined 35% and 37% respectively after 5 minutes of stimulation. These results that were obtained from the two popualtions of vesicles are related. This decrease in synaptic vesicle concentration approximated the decline in amplitudes of postganglionic nerve impulses which correlated as 1 to 1 with the two populations of vesicles in preganglionic terminals. These data points further evidence that transmitter is secreted in sympathetic ganglia by the exocytosis of synaptic vesicles and that the rate of transmitter secretion is dependent, in part, upon the availability of vesicles for release. (Supported by NIH Grant NS11325.)
NEUROCYTOLOGY


Axonal ultrastructure was studied in the magnocellular and parvocellular complexes of postmortem infant brainstem with the application of scanning electron microscopy (SEM). A correlative technique using SEM and the rapid Golgi method identified neurons and reticular processes in thirty-four infants ranging in age from 1 day to 8 months. In all cases the postmortem time did not exceed six hours. Suitable verification of apparent dendritic projections exhibiting spine-like extensions was established by examination of adjacent sections using light microscopy. These spine-like extensions were observed to be of the three morphological types described by Peters and Kaiserman-Abramof (1970). Stellate neuronal-like bodies were observed to be contained within the surrounding neuropil. In addition, neurites in close parallel apposition appeared to resemble a topographical synaptic arrangement. SEM analysis of the infant pons and medulla may provide new data concerning ultrastructural reticular correlates.


There are several biochemical and histological studies indicating that a deficiency of copper has severe consequences on the development of the nervous system in humans and other vertebrates. From these studies it seems that myelination is largely affected by copper deficiency although data from structural analyses are available. The purpose of the present work is to provide information on the role played by copper in the maintenance of the myelin sheath ultrastructure in optic nerve of adult rats. A group of 25 day old female rats was fed a copper-free diet and deionized double distilled water for 3 weeks. Control rats were fed the same diet and distilled water with 60 µg of copper added to it, during the same period. The optic nerves were processed for electron microscope examination and myelin was isolated from the whole brain. It was found that copper concentration in plasma and brain as well as a quantity of myelin isolated from copper-deficient rats were significantly less than those from controls. Concentration of copper in myelin isolated from copper deficient brains was slightly lower than controls but the difference was not significant. The electron microscope analysis showed advanced edema at the innermost region of the myelin sheath with marked axonal compression in 75% of the fibers analyzed. Edema seems to be formed by swelling of the inner loop with expansion of the myelin sheath which surrounds it. Edema is observed mostly in large diameter fibers. Small and medium diameter fibers showed only a slight swelling of the myelin inner loop. The results indicate that the innermost region of the myelin sheath is highly sensitive to copper deficiency and suggest that copper distribution may not be homogeneous within the myelin sheath. Supported partially by CONICIT Grant 31.36.01-0744.

1461 PUTATIVE TISSUE BASOPHIL/MAST CELL: A RESIDENT OF THE PIGEON OLFACTORY BULB. Karl E. Nieke, Daniel E. Bowers*, and Marvin S. Cannon*. Department of Anatomy, College of Medicine, Texas A&M University, Olin E. Teague Res. Center, College Station, Texas, 77843.

Small cells (8-10 µm) with a single nucleus and metachromatic granules have been observed in the various laminae of the olfactory bulbs of 16 pigeons (Columba livia). These cells have not been observed in the spinal cord, cerebellum, paleostriatal complex and the hyperstriatum. The cells were present in the bulbs of birds perfused through the heart (0.05 M phosphate and sucrose buffer, 3% glutaraldehyde, 1% paraformaldehyde, 600 mOsmol, pH 7.3) and in bulbs fixed by immersion. The presence of the putative tissue basophil or mast cell in the bulbs fixed by perfusion or immersion is important since forced diapedesis cannot account for their presence. The cells are rarely seen adjacent to blood vessels. Electron micrographs show these cells in the neuropil. Their cell surface possesses small pseudopodia, which suggest motility. By light microscopy utilizing blood smears and buffy coats, and Azure B staining, coarse granules within these cells stain metachromatically and average one micrometer in diameter. By electron microscopy, the granules appear membrane-bound and demonstrate a grid-like substructure. A second type of granule having a dense pleomorphic center surrounded by a pale zone, also is seen. The coarse granules may contain acid-mucopolysaccharide. In addition, histochecmic staining studies indicate that histamine may be present in these granules. Cells in blood smears and buffy coats of the pigeon that are identified as basophils closely resemble the cells seen in the olfactory bulb. Further histochemical studies on frozen sections of the bulbs and ultrastructural observations of blood basophils should clarify whether the cell observed in the bulb is the same as that seen in the peripheral blood.

Basophils or mast cells have not been described in the neu­

ropical or other avian species. Mast cells have been found in the brain of dogs and hedgeshogs. The putative basophil or mast cell in the pigeon bulb may have functions similar to those proposed for this cell in connective tissue or as yet undis­

closed functions.

1462 ABNORMALITIES OF AXODENDRAL MEMBRANE SPECIALIZATIONS IN MYELIN DEFICIENT "SHIVERER" MICE. J. Rosenbluth, Departments of Physiology and Rehab. Medicine, New York University School of Medicine, New York, N.Y. 10016.

The mouse mutant known as "shiverer" is characterized by a paucity of central nervous system myelin. Examination of thin sections of spinal cord, cerebellum and optic nerve reveals thin myelin sheaths with numerous irregularities. "Loops" of glial cytoplasm are apparent in various locations near the irregularly related to nodes of Ranvier. Although typical paranodal structures are uncommon, junctions containing "transverse bands" occur frequently in internodal regions and greater numbers of loose lamellae containing cytoplasm. Peripheral nerves exhibit subtle irregularities con­

sisting of myelin lamellae that terminate against the axolemma in internodal regions and greater numbers of loose lamellae containing cytoplasm. Peripheral myelin sheath thickness appears to be within normal limits, however. Analysis of freeze-fracture replicas of central nervous system fiber tracts shows few examples of typical paranodal membrane specializations in the E fracture face. However, distorted and bizarre junctional mem­

brane specializations occur frequently in the form of isolated patches or strips of membrane revealing the characteristic para­

crystalline pattern but oriented longitudinally or obliquely rather than nearly transversely. Occasionally several such strips, none of which encircles the axolemma, may occur side by side. The orientation of the strips may shift within a given region and the direction of the paracrystalline pattern within such a strip may also change abruptly. A few examples of typical E face nodal particle accumulations have been encountered adja­

cent to more or less normal paranodal or hemiparanodal regions. However, isolated paracristalline patches are usually not asso­

ciated with particle accumulations in the immediately adjacent membrane, except in instances where "lakes" are present. These are entirely surrounded by the paracrystalline membrane. The results indicate that even though myelin formation is grossly deficient in shiverer mice, the nerve cell membrane is not altered. In the central nervous system of Shiverer mice are still capable of forming the unique junctions normally found in the paranodal re­

gion. The lack of expression of E face particle accumula­

tions with isolated paracrystalline patches is compatible with the hypothesis that complete spiral collars of paracrystal­

line membranes are required for effective restriction of movement of intramembranous particles resulting in their local cen­

tration. Supported by grants from the National Institutes of Health and Muscular Dystrophy Association.

Periods of substantial Schwan cell proliferation occur during embryonic development and following peripheral nerve transaction or crush (Wallerian degeneration). The wave of proliferation observed during development is suspected to be stimulated by an axonal nitrogen (Wood and Bunge, 1975). By contrast, the increase in the number of Schwan cells in the distal transected nerve (Abercrombie and Johnson, 1946) may result from a breakdown process in which the axon becomes ensheathed and then is engulfed by the space vacated by the degenerating nerve fibers (Joseph, 1950). These suggestions would explain their observation that proliferation is greatest in degenerating myelinated nerves.

When rat dorsal root ganglia (DRG’s), treated with antimetabolites to suppress fibroblast outgrowth (Wood, 1976), are placed in tissue culture neurites elongate and become ensheathed by Schwan cells. Initially Schwan cells proliferate rapidly, but as each neurite is fully ensheathed division slows considerably and myelination commences. Using autoradiography to follow the incorporation of tritiated thymidine, we studied the effect of DRG exclusion (a. a. on Schwan cell proliferation. In the young explant (two weeks in vitro), as in the developing peripheral nervous system, Schwan cell proliferation is very high (labeling indices of 30% or more). In these cultures if the neuronal somas, which are confined in the ganglia, are passagionally pro- liferating rapidly decay to less than 10% only 48 hrs. later. In more mature cultures (six weeks in vitro), most cells were quiescent at the time of excision and remained quiescent indefinitely post-anoxia. However the myelinating Schwan cells (which could be recognized specifically by their intracellular myelin debris) did divide in response to axotomy. The peak labeling period occurred at four days post-axotomy at which time approximately 35% of the cells which had been myelin related, incorporated thymidine. These results suggest that the mitotic signals during development and degeneration are distinct. Breakdown of myelin debris or turnover of Schwan cell membranes may be the signal operative in Wallerian degeneration.

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Isolated Rat Brain Capillaries; Freeze-Fracture Confirmation of Inter-endothelial Junction Integrity. Richard R. Shivers and Gary W. Goldstein. Department of Zoology, University of Western Ontario, London, Ontario, and Department of Neurology, University of California Medical Center, San Francisco, California.

Fractions of rat brain tissue containing high concentrations of intact brain capillaries have been proposed as useful models for in vitro studies of blood-brain barrier permeability (Goldstein et al., J. Neurochem., 25, 1975). Preliminary studies of these preparations have suggested that the zonulae occludentes of the isolated segments of capillaries retain the impermeability to the protein tracer horseradish peroxidase exhibited by them in vivo. These junctions are therefore assumed to be functionally tight. We have utilized the precise structural organization of these occluding junctions, including an estimate of their tightness, to demonstrate a method of simple but precise assessment of junctional integrity, of pellets of isolated rat brain capillaries were freeze-fractured and then replicated with platinum and carbon. The freeze-fracture images of inter-endothelial zonulae occcludentes reveal complex intramembrane arrays of ridges and grooves characteristic of tight junctions. Longitudinal fractures of the cellular lining of capillaries expose vast expanses of inter-endothelial plasma membrane interfaces and the junctional complexes situated between the cells. From such images, the complex and elaborate architecture of the zonulae occludentes can be readily appreciated. Situated on PF fracture faces are 6-8 parallel ridges which are confined in the ganglia, are passagionally pro- liferating rapidly decay to less than 10% only 48 hrs. later. In more mature cultures (six weeks in vitro), most cells were quiescent at the time of excision and remained quiescent indefinitely post-anoxia. However the myelinating Schwan cells (which could be recognized specifically by their intracellular myelin debris) did divide in response to axotomy. The peak labeling period occurred at four days post-axotomy at which time approximately 35% of the cells which had been myelin related, incorporated thymidine. These results suggest that the mitotic signals during development and degeneration are distinct. Breakdown of myelin debris or turnover of Schwan cell membranes may be the signal operative in Wallerian degeneration.

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Large lysosomes containing wear-and-tear pigment are characteristic of neuronalftar and invertebrate. In Aplysia attention has been drawn to these particles because they are shown to be an Intracellular Ca+ sink and can release Ca+ in response to Ca+ stimuli. They are typically situated in a dense perinuclear layer together with the Golgi apparatus, smooth endoplasmic reticulum and vesicles in cell bodies of pigmented Aplysia neurons, which make chemical synapses with other neurons and with peripheral organs. We now have an indication that these lysosomes, in addition to being degradative in function, may also serve as a specific depot for transmitter substance in the cell body.

We have been studying the localization of 3H-serotonin injected directly into the cell body of the giant cerebral neuron (GCN), the identified serotonergic cell in the Aplysia cerebral ganglion, and have found a striking association of the labeled transmitter with large lysosomes. This localization is specific: 3H-serotonin was not found in similar lysosomes in the perikaryon of R2, an identified Aplysia cholinergic neuron.

3H-dopamine, 3H-histamine, and 3H-n-acteylglycineamine injected into the serotonergic neuron were not localized in lysosomal bodies. These organelles are indeed lysosomal: we showed that they contain acid phosphatase by electron microscope cytochemistry. Moreover, examination of isolated GCN cell bodies by fluorescence microscopy revealed the presence of abundant autofluorescent particles characterized by high fluorescence in the perikaryon. It is our working hypothesis that 3H-serotonin labels organelles membranes that once were components of the serotonergic vesicle. Lyosomes might accumulate the transmitter with a high degree of specificity because they contain membranes, perhaps recycled from the neuron's terminals, that retain in their ability to concentrate and bind serotonin. Alternatively the lysosomes may not take serotonin directly from the cytoplasm, but may engulf new serotonergic vesicles already charged with transmitter. Thus the lysosomes could regulate the supply of vesicles as well as the supply of the transmitter. Whatever the mechanism, lysosomal binding of exogenously introduced transmitter is an useful technique for identifying the neurotransmitter type of a neuron.


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1467 FURTHER NOTES ON THE SEARCH FOR "DOUBBLE" RETROGRADE AXONAL LABELING TECHNIQUES: "EVERYTHING BUT THE KITCHEN SINK." Dennis A. Steindler. Dept. of Anat., Michigan State University, East Lansing, MI 48824

Several macromolecules have been tested as potential "double-labels" to be used in combination with horseradish peroxidase (HRP) or determination of axonal branching within particular regions of the central nervous system. Previous investigators have reported retrograde axonal transport and subsequent axonal labeling with immunocytochemical techniques (e.g., anti-HRP, anti-synaptophysin, anti-adenosine, anti-HRP, anti-apo-HRP, 125-tetanus toxin, 125-hydroxyergosine) and confocal microscopy (e.g., Evans Blue), and iron dextran complex (Schwab and Kreutzberg, '75; Gest et al., '76; Hayes and Rustioni, '78; Price et al., '77; Schwab et al., '78; Kristensson and Olsson, '78; Steward and Scoville, '76; Koppers et al., '77; Olsson and Kristensson, '78).

This investigator has injected various dyes, iron dextran complexes, tritiated RNA precursors (i.e., uridine, hypoxanthine, alloxanthine), alkaline phosphatase, and labeled WGA in different regions of mouse cerebellum, brain, and basal ganglia. Preliminary observations are encouraging using lactic acid (Figure 1) which was either titrated to pH 3.8 or neutralized with NaOH. The mechanisms of uptake and transport are not known, but the sequence most likely begins with WGA binding to specific glycoproteins and glycolipids located on the cell surface (Schwab et al., '78). In addition to autoradiography, there are also several alternatives for histochemically demonstrating the lectin, any of which use various or substituted carbohydrates. WGA (M.W.35,000) bound to alkaline phosphatase (M.W.140,000) will hydrolyze the phosphate group of 5-bromo-4-chloro-3-indolyl phosphate producing an insoluble blue indigoid dye (Chu, pers. comm.; Tsou et al., '67) that is compatible with a brown reaction product using 3,3' diaminobenzidine tetrahydrochloride in HRP histochemistry. WGA bound to biotin (E.Y.Laboratories, San Mateo, Ca.) and horseradish peroxidase (E.Y.Laboratories, San Mateo, Ca.) or biotin (E.Y.Laboratories, San Mateo, Ca.) and alkaline phosphatase (Sigma) or biotin (E.Y.Laboratories, San Mateo, Ca.) and tritiated RNA precursors (i.e., uridine, hypoxanthine, alloxanthine) may afford better uptake and transport because of the considerably smaller molecular weight of biotin compared to AP, and sections can be reacted with wodan (which displays an extremely high binding affinity for biotin) bound to either AP or ferritin for light and electron microscopic detection of the transported lectin. Analysis of degree of divergence and collateralization projections within the motor system will follow the following refinements of the aforementioned procedures.

(Supported by NIH NSRG RR05772-04.)


Sciatic nerves of frog tadpoles were fixed and freeze-fractured, and complementary replicas of each face were prepared. At least 12 nerve segments from different regions of each nerve were prepared and analyzed. The results confirm a previous study of amphibian myelinated fibers in the central nervous system (J. Neurocytol. 5:731) in showing essentially high concentrations of intramembrane particles in the E face and F fracture faces of the nodal axolemma but low concentrations of E face particles in the internal nodal region relative to the internodal F face particle concentration. By comparing complementary replicas it is possible to demonstrate unequivocally that a large proportion of the nodal E face particles are large in diameter and cast long shadows, while in the F face fracture the proportion of large particles is distinctively lower and many small particles and irregularly-shaped structures are present. Tracings of E and F fracture faces of the nodal and paranodal axolemma and of the junctional Schwann cell membrane were prepared and superimposed to face in order to determine whether the particles in the respective tracings overlap. Almost none do, indicating that the particles in the respective fracture faces are distinct from each other and do not represent fragments of the same unit. Any particles that do overlap, they may be members of the same population, most of which remain attached to the F face in the cleaving process. Supported by grants from the National Institutes of Health and the National Institutes of Health and the National Institutes of Health.


Bipolar specimens of omental veins were obtained during surgery under general anesthesia. These were normotensive female patients who had not been exposed to any medication known to release histamine. The excised veins were divided into segments, mounted in a bath and subjected to electrical field stimulation at 1 Hz for 60 sec with or without the Ph receptor blocking agent, phentolamine (7.5 x 10^-8 M) present. Control segments were superfused for the length of time of the experiment. Small tissue blocks from several areas of all segments were prepared for electron microscopy. Noradrenergic nerve terminals with typical large and small dense-cored vesicles and mast cells with large characteristic scroll-containing granules were well preserved and easily identified in control veins. The distance between mast cells and noradrenergic axons and terminals was frequently in the same range as that between nerve terminals or mast cells and smooth muscle cells, i.e., 1 μm or less. After electrical stimulation without phentolamine present there was an increase of presumptive exocytotic profiles along the terminal membrane and an increase in the total number of small vesicles by 30% when compared with unstimulated controls. There was no obvious change in the mast cell ultrastructure. However, after stimulation in the presence of phentolamine, both nerve terminals and mast cells showed morphological changes. In the nerve terminals there was a significant increase of both small and large vesicles that were in immediate contact with the terminal membrane. The total number of large and small vesicles had doubled when compared to superfused controls but there was an increase of small clear vesicles. In the mast cells, many of the intracellular granules had fused and showed decreased electron density. Granules and released vesicles were present extracellularly. Anastomosing collagen-filled cavities filled with amorphous material were prevalent in other mast cells. It is possible that this degeneration of the mast cells following electrical stimulation in the presence of phentolamine reflects a direct releasing effect of a compound which is structurally reminiscent of histamine. It may also be due to an indirect effect involving noradrenergic and ATP overflow after blockade of the prejunctional Ph-adrenergic receptors.

(Supported by USPHS GM 15490.)


A system of acidic macopolysaccharides is present in the lateral wall and angle of the lateral ventricle of the young adult rat. This macromolecular system appears to be present from the early postnatal period through adulthood. This macropolyelectrolyte system, which can be identified in cryostat sections, this network is not extracted by chloroform/methanol (2:1) or xylene. Carboxylic aldehydes such as paraformaldehyde reveal the network, and negative staining with toluidine blue at pH 1.5 will block staining. Staining with alcian blue at pH 1.5 will block staining. Staining with alcian blue at pH 1.5 will block staining. A system of acidic macopolysaccharides has been demonstrated in the brain of young adult rats has been demonstrated by means of histochemical and electron microscopic preparations. This extracellular macromolecular network appears to be present in the lateral wall and angle of the lateral ventricle at these sites which show the immediate subependymal region. The extracellular matrix network appears to be present in the lateral wall and angle of the lateral ventricle. The extracellular matrix network appears to be present in the lateral wall and angle of the lateral ventricle. In addition to demonstrating the presence of macropolyelectrolytes in the ventricular extracellular space at these sites, which should be important in trans-ependymal ionic movement.
NEURO-ENDOCRINOLOGY
1471 EFFECTS OF CASTRATION ON DOPAMINE METABOLISM IN RAT STRIATUM AND LIMBIC FOREBRAIN. Lloyd M. Alderson*, Matthew S. Starr*, and Michael J. Baum. Department of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

Psychopharmacological studies have suggested that increased activity in dopaminergic pathways facilitates the expression of sexual behavior in the male rat. Castration of the male rat leads invariably to the disappearance of masculine sexual behavior. Experimental studies have examined whether or not a castration-dependent reduction in copulation is correlated with changes in catecholamine synthesis or in dopamine (DA) metabolism in brain regions which contain DA-containing nerve terminals.

In an initial experiment catecholamine synthesis was estimated in castrated and sham-operated adult male rats (Long-Evans) by measuring the accumulation of dihydroxyphenylalanine (DOPA) by radioenzymatic assay 30 min after injection of NBI-1015 to inhibit aromatic L-amino acid decarboxylase activity. Males were killed at night either 15 or 30 days after castration or sham-operation. The levels of DOPA measured in striatal slices and in limbic forebrain (Nucleus Accumbens plus lateral septal region) were identical in castrated and sham-operated males at both postoperative times. Thus we were unable to confirm the report (Engel et al., 1979, Pharm. Biochem. Behav. 10:149) of increased DOPA accumulation in each of these neural tissues after castration.

Dopamine metabolism was studied in striatum and limbic forebrain in male rats. Since DOPAC and homovanillic acid (HVA) using high pressure liquid chromatography. Males were again killed 15 or 30 days after castration or sham-operation. In stratum the levels of DOPAC were significantly lower in castrated males 30 days post-operatively. Stratal HVA also tended to be lower in castrates than in sham-operated rats at both times, although this difference did not reach statistical significance. In limbic forebrain no significant differences between castrated and sham-operated males were detected in DOPAC or HVA, although levels tended to be lower in castrates both 15 and 30 days postoperatively. The results are consistent with the notion that testicular steroids, or their neural metabolites, normally facilitate the release of dopamine in the male rat striatum and thereby contribute to the activation of sexual behavior.


Biochemical fluorescent techniques have revealed that the neurointermediate lobes (NIL) of the rat pituitary contains catecholaminergic nerves (Björklund, Z. Zellforsch. 82: 573, 1968). Dopamine (DA) nerves terminating in the NIL originate in the arcuate nucleus, a nucleus located in the hypothalamus which projects to the pituitary gland. It has been suggested that the arcuate nucleus is involved in the regulation of the NIL.

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It has been shown that serotonin (5HT) may play an inhibitory role in the control of sexual receptivity in the rat. Since all known serotonergic neurons have their cell bodies within the brainstem raphe nuclei, the effects of bilateral electrical stimulation of the mesencephalic raphe nuclei and adjacent periaqueductal grey (PAG) on lordosis behavior were investigated, using four different sets of stimulation parameters. Unilateralized, serotoninergic animals had been prepared in one of these sets. In our experiments the concentration of DOPA in various brain regions was estimated by determining the rate of accumulation of DOPA in NIL should be appropriate for estimating the activity of the mesencephalic dopaminergic neurons.

Dopaminergic nerves terminating in the NIL originate in the arcuate nucleus, a nucleus located in the hypothalamus which projects to the pituitary gland. It has been suggested that the arcuate nucleus is involved in the regulation of the NIL.

In castrated male zebra finches (Poephila guttata), the autoradiographic method was used to determine the distribution of hormone accumulating cells in selected brain regions after injection of tritiated testosterone (T), dihydrotestosterone (DHT), or estradiol (E). The brain regions selected for analysis showed extremely vigorous hormone uptake. The autoradiograms revealed that T and E injected in different areas of the brain showed cellular accumulation of hormone after T injection. These results, when taken together, suggest that T or its non-aromatized metabolites are present in the brain, and that these may be important in the regulation of behavior.


In castrated male zebra finches (Poephila guttata), the autoradiographic method was used to determine the distribution of hormone accumulating cells in selected brain regions after injection of tritiated testosterone (T), dihydrotestosterone (DHT), or estradiol (E). The brain regions selected for analysis showed extremely vigorous hormone uptake. The autoradiograms revealed that T and E injected in different areas of the brain showed cellular accumulation of hormone after T injection. These results, when taken together, suggest that T or its non-aromatized metabolites are present in the brain, and that these may be important in the regulation of behavior.
THE IDENTIFICATION AND QUANTIFICATION OF 1,2,3,4-TETRAHYDRO-β-CARBOLINE AND 6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE IN RAT BRAIN AND ADRENAL GLAND, S. A. Barker and R. E. Harrison (Spon: J. A. Monte), Neurosciences Program, University of Alabama in Birmingham, B’ham, AL 35294.

GC/MS analyses of rat brain and adrenal gland extracts have led to the identification of 1,2,3,4-tetrahydro-β-carboline (THBC) and 6-methoxy-THBC (6-MeO-THBC) as naturally occurring constituents of these tissues. Trace amounts of 2-methyl-THBC (2-MTHBC) were also identified.

Rat brains and adrenal glands from individually housed animals were homogenized and spiked with deuterated standards for 6-MeO-THBC and THBC. The samples were extracted and derivatized to form the corresponding heptafluorobutyryl compounds (Barker et al., Biochem. Biophys. Res. Commun. 97:146-154, 1979). Analyses were conducted by selected ion monitoring, comparing ion mass ratios and retention times of the added deuterated standards with peaks for the endogenous β-carbolines.

THBC 6-MeO-THBC 2-MTHBC

BRAIN

17.5 ± 4.86 ng/g
325 ± 45 ng/g
1113.7 ± 300 ng/g

ADRENAL

35.6 ± 16.6 ng/g
1113.7 ± 300 ng/g

TRADE

The implications of these findings, in view of the known pharmacological effects of these compounds on amine uptake and monoamine oxidase activity, will be discussed.


Electrolytic ablation of anteroventral third ventricle (AV3V) periventricular tissue results in temporary adipsia, chronic hyponatremia and expanded blood volume, suggesting that mechanisms of sodium regulation are altered. The present experiments were designed to determine if AV3V lesions change sodium consumption under ad lib conditions and the natriuresis typically seen following isonicotic blood volume expansion.

Rats were maintained on sodium deficient chow to 2.0% NaCl solution and water. Animals were evaluated for sodium solution intake and received either electrolytic lesions in the AV3V region or sham lesions. NaCl solution and water intakes were recorded 16 days postsurgery and following the hyponatremia induced by s.c. Formalin. Separate groups of animals underwent AV3V ablation or sham lesions. These animals were implanted with venous, arterial and bladder catheters, and isotonic NaCl solution was infused intraventrically at .51 ml/min until 10% of body weight was given. Urine and sodium excretion was measured during the infusion period. In addition, blood samples were taken, pooled and bioassayed for natriuretic hormone according to the procedures of Gruber and Buckalew (Proc. Soc. Exp. Biol. Med., 159:463-467, 1978).

Animals with lesions surrounding the AV3V consumed significantly less sodium solution and showed lower sodium preference following surgery than sham lesioned animals. However, both groups increased sodium consumption following sodium solutions. These data indicate that AV3V lesions attenuate sodium intake but do not render animals insensitive to their sodium levels.

In addition, AV3V lesioned animals showed markedly attenuated urination and sodium excretion compared to control animals during volume expansion. The lack of sensitivity to natriuretic stimuli in the AV3V lesioned animals might reflect a general impairment of renal function. These data indicate that AV3V lesions reduce the ability of animals to conserve sodium and urinate in response to sodium deficiency.

A SEX DIFFERENCE IN THE ENDOGENOUS RELEASE AND IN THE EFFECT OF MONOA MINE OXIDASE ACTIVITY, WILL BE DISCUSSED. A. J. Crowe and W. S. Yeh, depts. of Neurology and Physiology, Univ. of Minnesota, Minneapolis.

Biology Program, Univ. of Illinois, Urbana, IL 61801.

Holtzman rats were decapitated at the same time each afternoon, the anterior pituitary gland removed and immediately placed in ice cold Krebs Ringer phosphate buffer glucose-BSA medium at pH 7.4. Pituitary glands were quartered and placed into individual perifusion chambers, perfused with a modified Krebs-Ringer glucose-BSA medium and maintained in a 37°C water bath. The medium was pumped into the chambers at a constant rate of 1 ml/4 min. After a 1 hour stabilization period perfusate was collected in 4 min intervals on ice. The samples were stored at -20°C until the RIA's were performed.

The release of endogenous prolactin from the male pituitary gland was 3.6 ± 0.67 ng/g at 37°C and 11.3 ± 1.5 ng/g at 5°C. In contrast, the release of endogenous prolactin from the female gland was 2.1 ± 0.1 ng/g at 37°C and 1.3 ± 0.1 ng/g at 5°C. There was no significant difference in the release rates of prolactin between male and female pituitaries. In contrast, the release of endogenous prolactin from male and female pituitaries was stimulated by AMT. The release rate of prolactin from male glands (n=6) increased from 2.1 ± 0.1 ng/g at 5°C to 4.1 ± 0.6 ng/g at 37°C. The release rate of prolactin from female glands (n=6) increased from 2.7 ± 0.1 ng/g at 5°C to 3.0 ± 0.1 ng/g at 37°C. The release of endogenous prolactin from male and female pituitaries was not stimulated by AMT at 5°C.

Prolactin secretion from male glands (n=6) went from 1.13 ± 0.1 ng/g at 37°C to 11.5 ± 1.5 ng/g at 37°C. The release rate of LH in females was, diestrus: 2.7 ± 0.3 ng/mg/min at 0°C, 6-methoxy-THBC (6-MeO-THBC) as naturally occurring constituents of these tissues. Trace amounts of 2-methyl-THBC (2-MTHBC) were also identified.

Formalin. These data indicate that AV3V lesions attenuate sodium intake but do not render animals insensitive to their sodium levels.

In addition, AV3V lesioned animals showed markedly attenuated urination and sodium excretion compared to control animals during volume expansion. This could be the result of impaired release of natriuretic hormone. These studies suggest that the integrity of the AV3V periventricular tissue is essential for normal sodium regulation.


Electrolytic ablation of anteroventral third ventricle (AV3V) periventricular tissue lesions ingest less sodium than controls while on sodium deficient chow, but remain sensitive to body sodium concentrations. In addition, rats with AV3V lesions have a lower body weight for natriuresis when volume expanded. This could be the result of impaired release of natriuretic hormone. These studies suggest that the integrity of the AV3V periventricular tissue is essential for normal sodium regulation.

This is a sex difference in the endogenous release and in the effect of temperature on the release of prolactin and LH from the anterior pituitary of rats in perifusion. Deborah Beaure* and V.D. Ramirez (Spon: R. Gliette), Dept. of Physiol. and Biophys. and Neural and Behavioral Biology Program, Univ. of Illinois, Urbana, IL 61801.

Holtsman rats were decapitated at the same time each afternoon, the anterior pituitary gland removed and immediately placed in ice cold Krebs Ringer phosphate buffer glucose-BSA medium at pH 7.4. Pituitary glands were quartered and placed into individual perifusion chambers, perfused with a modified Krebs-Ringer glucose-BSA medium and maintained in a 37°C water bath. The medium was pumped into the chambers at a constant rate of 1 ml/4 min. After a 1 hour stabilization period perfusate was collected in 4 min intervals on ice. The samples were stored at -20°C until the RIA's were performed.

The average release rate of prolactin from female glands was, diestrus: 2.7 ± 0.3 ng/g; preproestrus: 3.98 ± 0.27 ng/g; and estrus: 4.6 ± 0.22 ng/g. The average rate of prolactin release from male glands was 2.13 ± 0.10. The release rate of LH in females was, diestrus: 2.7 ± 0.3 ng/mg/min at 0°C, and 6-methoxy-THBC (6-MeO-THBC) as naturally occurring constituents of these tissues. Trace amounts of 2-methyl-THBC (2-MTHBC) were also identified.

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Chemical stimulation has been used in numerous studies of catecholamine secretion from slices of adrenal medulla. However, chemical stimulation cannot simultaneously excite all of the cells in the slice because of the time required for diffusion of the secretogogues to the site of the stimulus. In contrast, electrical stimulation should simultaneously excite all of the chromaffin cells in the tissue slice. Therefore, we tested the electrical stimulation of rat and guinea pig chromaffin cells for kinetic experiments involving stimulation-secretory processes, including ion movements, protein synthesis and catecholamine synthesis and release.

The studies reported here have been designed to determine if electrically-induced catecholamine secretion is real, and not a consequence of cell damage or secretion secondary to stimulation by 27%. Thus, approximately 75% of the electrically-induced catecholamine secretion was due to direct stimulation of the chromaffin cells, the remainder being secondary to acetylcholine release by cholinergic terminals presynaptic to the tissue. Acetylcholine-induced secretion from chromaffin cells is dependent upon extracellular calcium concentration. With electrical stimulation in calcium-free medium, secretion was reduced by only 1% compared to stimulation in complete or calcium-enriched media. Thus it appears that sufficient calcium for a normally secretory response is available from intracellular compartments.

Based upon these results, electrical stimulation may be a useful means of evoking secretion. (Supported N.I.H. 5506 RR 0976-01).
appears that the changes in excitability produced by using in vitro slices from male hippocampus. It thus synaptic EPSPs and population spikes in the CA1 subfield. This effect was reflected in steepened slopes of the NL, and numerous axon terminal sprouts occur within these axons. In rats rendered adipsic by AV3V lesions, most neuronal somas in the SON resemble those of sham lesioned animals, although occasional cells show accumulated NGV. Degenerating axons are common, and nerve terminals undergoing degeneration are present in axodendritic synapses and synapses on neurosecretory somata. The sizes of the somas, their nuclei and especially nucleoli are increased, endoplasmic reticulum is hypertrophied and more often dilated and a striking accumulation of NGV is present. The neuron terminals are degenerating and being phagocytosed by microglia. Responses to osmotic stimuli and to angiotensin are abolished or occluded by zona glomerulosa lesions. In rats with adipsic lesions, it is likely that degenerating axons and terminals present in SON of lesioned rats are processes of osmoceptors and angiotensin receptors, the somas of which are damaged by the adipsic lesions. Consequently, in this study the AV3V-degenerating neurons were not stimulated by the severe dehydration to release their NGV. Their accumulation in the NL indicates that synthesis and transport of NGV is impaired in this time period. Supported in part by ISHBP 405 2302, 180 1 NS 14062 and NIH 1 E 02 0 M 0 0 0 0 6 .


In a prior experiment we observed gender-related changes in the stability of CA1 pyramidal cells in isolation when gonadal steroids were administered to hippocampal slice preparations (Vardaris & Tevlier, 1978, Neurosci. Abstr., no. 4, 253). Enhanced autoradiographic potentials were obtained from male slices 10 and 20 minutes after exposure to 100 nM 17beta-estradiol in the superfusion fluid. The present study was designed to determine whether similar effects occur in the intact preparation. Male rats were curarized and respiration with room air and metofane gas during stereotactic surgery. Wound margins and pressure points were procainized. Final placement of the recordings micropipette tip in the cell body layer of CA1 and the tip of the steel stimulating electrode in the Schaffer collateral system was verified electrophysiologically through laminar analysis. A presteroid input/output function was recorded 40 minutes after withdrawal of general anesthesia. Stimulus voltages were selected to permit recordings of extracellular EPSPs as well as population spikes. The presence of paired-pulse facilitation and recurrent inhibition were used as criteria for appropriate physiological status of the preparations.

One hundred and 200 microampere pulses of 17beta-estradiol benzoate were administered by intraperitoneal cannulation. Input/output functions were obtained 20, 40, and 60 minutes after infusion of the sex steroid. The threshold of stimulus intensities was used in all input/output function determinations. Analysis of the recorded potentials revealed that both dosages increased the amplitudes of mono-synaptic EPSPs in the CA1 population field. This effect was reflected in steepened slopes of the post-stereoid input/output functions with no significant changes in threshold values. These results are consistent with our findings using in vitro slices from male hippocampus. It thus appears that the changes in excitability in estradiol can be obtained from intact preparations in the presence of normal metabolism.
EFFECTS OF SEPTAL LESIONS WITH KAINIC ACID IN PREPUBERAL FEMALE RATS ON THE RELEASE OF LUTEINIZING HORMONE AND THE ONSET OF PUBERTY. Richard U. Colombo and James P. Nies, Department of Anatomy, University of South Florida, Tampa, Florida 33612.

The injection was completed in 1 minute. Thirty minutes later, [14C] 2-deoxyglucose was injected in 0.5 ml of saline.

In order to test whether the septal area was involved in the normal onset of puberty in the female rat, a different group of females was used and were monitored daily for vaginal opening (VO) and vaginal smear cyclicity. KA lesioned animals showed a delayed onset of VO in contrast to that of control rats. Sham-lesioned rats had a slight delay in the onset of their VO, probably due to non-specific damage by the vehicle saline infused into the septal area. Vaginal cyclicality was normal in all animals in both groups. All rats showed a relative lack of sperm in seminal plug. These results suggest that intrinsic septal neurones are involved in the oestrogenization of the positive feedback of oestrogen on LH release. In addition, our results show that the normal somatic development of the female rat is altered by KA injection of the septal area and the development of events that lead to the onset of adult female reproductive ability. Supported by NIH grant HD-11011 and NSF RIA-SERT-06922.

LOCAL INCREASE IN THE UPTAKE OF [14C] 2-DEOXYGLUCOSE AFTER ELECTROCHEMICAL DEPOSITION OF IRON IN THE BRAIN. J.A. Colombo and S. Saporta, Department of Anatomy, College of Medicine, University of South Florida, Tampa, Florida 33612.

Since the original description by Everett and Radford (1961), electrochemical stimulation technique has been widely used in the field of neuroendocrinology. It has been assumed that delivering anodic direct current through a stainless steel electrode increases local neuronal activity, and that this increase in activity is mediated through the release of iron ions into the surrounding extracellular space. One question that has been shown that multimodal activity recorded within the area of iron deposition undergoes a significant increase (Colombo et al., 1974; 1975). We now report evidence that electrochemical stimulation will increase the uptake of [14C] 2-deoxyglucose within the area of iron deposition in the brain. Adult male rats were anesthetized with pentobarbital and an indwelling catheter placed in one femoral vein. A pair of electrodes were lowered stereotactically into the preoptic suprachiasmatic region. The electrode pair consisted of an insulated 00 stainless steel insulated stainless steel electrode and a 125µm teflon insulated platinum wire. The electrodes were allowed to stabilize for 10 minutes before the onset of 30µA ADC stimulation delivered for 30 sec sequentially through each electrode. Three to four minutes after stimulation, a bolus of 400µCi/100g body weight of [14C] 2-deoxyglucose was injected in 0.5 ml of saline. The injection was completed in 1 minute. Thirty minutes later, the local DC current was turned off. The brain sections (20µm) were taken alternately for either autoradiography or for identification of the area of iron deposition with Gamo's iron stain. The sections were exposed to x-ray films for 6 days. Autoradiographs show a clear increase in the concentration of the radiolabeled 2-deoxyglucose which overlaps with the area of iron deposition. A comparatively reduced cox4-contour for 2-deoxyglucose is visible immediately surrounding the lesioned area at the tip of the platinum electrode. These results indicate that iron deposition produces electrochemical means results in increased local metabolic activity of the exposed neuronal population. Experiments with [3H] 2-deoxyglucose are currently underway in order to analyze this effect in a more quantitative manner.

Partially supported by Biomedical Research Support Grant S 507 8805749, Division of Research Resources, NIH.
RAT PARS INTERMEDIA ACTION POTENTIALS AND THEIR RESPONSE TO VARIOUS AGENTS.

M. Huff Davis* and Audrey Garbman* (SPON: P. E. Pickens).
Department of General Biology, University of Washington, Seattle, Washington 98195.

Action potentials have recently been recorded from a number of endocrine glands and their tumors. It appears that this electrical activity is related to cell secretion. Prolactin, from the pars distalis, and GH, from the pars intermedia, have several phenomena in common. They both respond to similar releasing or inhibiting factors of hormone release, both spontaneously secrete in the absence of hypothalamic control, and action potentials can be recorded from either one. Thus, data obtained from prolactin secretion seems to fit well with GH secretion and vice versa. There is difficulty in obtaining information on electrical activity from the pars distalis because of its heterogeneous population of cells. One has a hard time defining exactly which cell is being recorded from and consequently must usually rely on tumors. The pars intermedia lobe has the advantage of having relatively few cell types and presents a better target for electrophysiological studies.

Following the decapitation of 250 gram rats (SD), neurointermediate lobes, containing the pars intermedia and pars nervosa complexes, were cannulated and placed in a container with Kreb's Ringer. The preparation was then gassed in a 95% O2:5%CO2 mixture. Glass microelectrodes, filled with 4M NaCl, were used to record extracellular potentials. When a cell with spontaneous electrical activity was found, various agents were added to the surrounding media of any changes observed. At a 10^-5 concentration of isoproterenol, a rapid increase in spike frequency was found, lasting several minutes. High potassium (10X) and norepinephrine (10^-5), on the other hand, caused a decrease in spike frequency and, in some cases, eliminated the activity all together. The latency between the application of experimental solutions and the effect in any given experimental series. Therefore, agents which enhance or reduce the release of GH are correlated with an increase or decrease in the frequency of action potentials in the pars intermedia.

(U.S.P.H.S. Grant AM-16282)


Intense and frequent male aggression is seen when a strange male rat is placed into a small colony containing resident male and female rats. The intensity and frequency of aggression between male and female colony residents in response to male and female intruders and the importance of hormonal condition for attack. In small colonies, we were able to separate the two large groups of male rats and two females. After each colony began producing litters they were tested for response to a male intruder. Within a few weeks one of the females in each colony reliably attacked male intruders. This alpha male and one of the two females were then tested together biweekly for response to male and female intruders. Male residents were highly aggressive toward castrated, castrated and testosterone propionate (TP) treated male intruders; less male aggressive behavior was seen toward castrated intruders treated with estradiol benzoate or only TP treated females. Residents rarely attacked or mounted female intruders independent of the hormonal condition of the females. Female residents, on the other hand, attacked castrated male intruders which had been treated with EB or EB + P. Female attacks toward EB or TP treated females was not correlated with the females' reproductive condition (e. g. pregnancy, lactation). In a second series of experiments the importance of the male's reproductive condition was examined. Half of the resident males and females were gonadectomized and then tested for aggressive behavior for 7 weeks post-surgery. The results indicated that male residents declined slowly after castration. The rate of decline in male aggressive behavior by castrates was similar to the decline of their mounting of receptive females. Therefore, agents which enhance or reduce female aggression were somewhat more aggressive than normally intact females. These experiments demonstrate that both male and female rats can be highly aggressive, but that the behavior is essentially an autonomic, both in terms of the characteristics of the most effective stimuli and in the gonadal control of the behavior. The hormonal determinants of male and female aggressive behavior are obviously quite different.

This study was supported by USPHS Research Grant DA-1502.

AGGRESSIVE BEHAVIOR IS INHIBITED IN MALE BUT NOT FEMALE RATS TREATED WITH 6-OHDA IN THE LEVERS


The role of central dopaminergic neurons in the regulation of prolactin (PRL) secretion has been studied extensively. Alterations in dopaminergic function (which have been implicated in the pathogenesis of schizophrenia) are reflected in changes during and two hours postinfusion. Six patients were infused on pimozide and the procedure was repeated twice, one and four weeks following treatment discontinuation. In normal subjects, serum PRL was significantly higher in patients on pimozide compared either to controls or to the same patients off pimozide.

The latency between the application of experimental solutions and the effect in any given experimental series. Therefore, agents which enhance or reduce the release of GH are correlated with an increase or decrease in the frequency of action potentials in the pars intermedia.


The rate of accumulation of DOPA in the striatum (ST) following the administration of a decarboxylase inhibitor has been used to estimate the activity of nigrostriatal dopaminergic neurons (Carlsson et al. J. Pharmocol. Biochem. 5, 1974). In this study, we undertook to determine if DOPA accumulation in the median eminence (ME) could be used to estimate the activity of the tuberoinfundibular DA neurons. Administration of DOPA in ME and ST of the rat was determined 30 min after the administration of 3-hydroxybenzylhydrazine (NSD 1015; 100 mg/kg, i.p.) by a radioenzymatic assay sensitive to 50-125 pg of DOPA. In this assay tissue extracts containing DOPA are incubated with catechol-O-methyltransferase and 3-hydroxydopamine and the radioactive product, 3-methoxytyrosine, separated by ion-exchange chromatography and charcoal adsorption. The ME, unlike the ST, contains appreciable amounts of norepinephrine (NE), the most important inhibiting factor of hormone release, both spontaneously secreting cells of the pars distalis because of its heterogeneous population of cells. The latency between the application of experimental solutions and the effect in any given experimental series. Therefore, agents which enhance or reduce the release of GH are correlated with an increase or decrease in the frequency of action potentials in the pars intermedia.
EFFECTS OF ESTROGEN TREATMENT ON NORMAL AND SENSITIZED RAT STRIATAL DOPAMINE (DA) RECEPTORS AND DA-SENSITIVE ADENYLYL CYCLASE. D. Gi Paolo*, P. Labrie*, A. Dupont*, N. Barden* and P. Tanguay* (SPON: G. Radouce-Thomases). Laboratory of Molecular Endocrinology, CHU, Québec, Québec, G1V 4G2, Canada.

Estrogen treatment has recently been found to exert potent antiapaminergic activity at the pituitary and sexual levels. In such treated animals, plasma levels of prolactin increase, and plasma levels of somatomedins (IGF-I and IGF-II). In addition, estradiol decreases both the synthesis and the release of prolactin into the circulation. In the present study, we have examined the effect of estrogen treatment on the number of DA receptors in the striatum of normal and sensitized rats. The results indicate that estrogen treatment decreases the number of DA receptors in the striatum of normal and sensitized rats.

HYPERPROLACTINEMIA AND MATING BEHAVIOR IN THE MALE RAT: EFFECTS OF PREVIOUS SEXUAL EXPERIENCE AND BROMOCRIPTINE TREATMENT. P.C. Doherty, Jr.*, A. Bartke*, and M.S. Smith*. (SPON: N. Hagino)

Estrogen treatment has been found to suppress mating behavior in male rats. This effect is greater in sexually experienced males than in inexperienced males. We have investigated the effect of bromocriptine on the mating behavior of male rats. Bromocriptine was administered to male rats, and the effect on mating behavior was assessed. The results indicate that bromocriptine increases the number of mounts and the duration of copulation in male rats treated with estrogen. These effects are reversed by the administration of a DA receptor antagonist. These results suggest that bromocriptine may act as an agonist at the DA receptor and that the effect of estrogen treatment on mating behavior is mediated by changes in DA receptor activity.

CORTICOSTEROINE AND RNA METABOLISM IN THE RAT HIPPOCAMPUS. Linda A. Doka*, J.R. M. J. Walker*, and M.C. Ward*. (SPON: H. J. Waller), Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699

Recent reports have provided evidence that corticosteroids may have direct biochemical effects upon the hippocampus. Corticosterone, the functional glucocorticoid in the rat, enhances the synthesis of a particular hippocampal protein of 54,000 daltons (Miller, et al., Psychoneuroendocrinology 3:155). Since autoradiographic evidence suggests that effects of corticosterone in the hippocampus are mediated by nuclear localization of the steroid with subsequent incorporation of [3H]-uridine into RNA, the present study was initiated to characterize corticosterone metabolism in the rat in response to corticosterone. The hippocampus was removed from anesthetized adult male Sprague-Dawley rats before 11 a.m. to minimize the endogenous levels of corticosterone in the animals. Each hippocampus was halved bilaterally and divided between Hepes-salts-glucose media with and without 1 x 10^{-5}M corticosterone. Following incubation, the tissue from 4 animals for 1.5 hrs 1 steroid, 100µCi of [3H]-uridine was added to each incubation for an additional 2.5 hrs. Incorporation of the [3H]-uridine into RNA was measured as total TCA-precipitable radioactivity/mg protein, 85% of which was RNA-sensit. The uptake of [3H]-uridine into hippocampal tissue was determined as TCA-soluble radioactivity/mg protein. Incubation with corticosterone decreased the labeling of the hippocampal TCA-precipitable radioactivity. The labeling of hippocampal TCA-precipitable radioactivity was 10% lower than that seen in control incubations. In spite of this reduction in the labeled RNA precursor pool, corticosterone-treated hippocampal tissue showed incorporation of [3H]-uridine into RNA, the present study was initiated to characterize corticosterone metabolism in the rat in response to corticosterone.

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CHANGES IN LOCOMOTOR ACTIVITY ASSOCIATED WITH THE PHOTOPERIODIC RESPONSE OF THE TESTES IN MALE GOLDEN HAMSTERS. Gary R. Ellis* and Fred W. Turek (SP能: C. E. nch-ougi). Dept. of Biological Sciences, Northwestern University, Evanston, IL 60201.

The light-dark environment is known to be a major environmental signal entraining circadian rhythms of locomotor activity. Recent evidence indicates that steroid hormones also influence circadian activity rhythms. The precise contribution of such external (e.g., photoperiod) and internal (e.g., hormones) environmental factors in regulating activity are not well defined. In order to examine the effects of the short photoperiod on circadian changes in the reproductive system upon locomotor activity, male golden hamsters were exposed to either 14 h of light per 24 h (LD 14:10) or to 6 h of light per 24 h (LD 6:18). Locomotor activity was recorded continuously, and testicular size and serum testosterone were measured periodically throughout the study. The short photoperiod induced a decrease in testicular weight, serum testosterone, and the number of wheel revolutions per 24 h period, when compared to the levels of these variables exhibited by LD 14:10 hamsters. Prolonged exposure to LD 6:18 resulted in a spontaneous increase in these three parameters to levels indistinguishable from those observed in LD 14:10 animals.

The LD 6:18-induced testicular regression was an increased lability of the time of daily activity onset. A return to stability in the day-to-day time of activity onset was coincident with spontaneous testicular regrowth in LD 6:18 hamsters. Exposure to LD 6:18 induced an expanded activity time. This increased duration of the daily active phase persisted even after the spontaneous increase in testicular weight, serum testosterone, and the number of wheel revolutions per 24 h period, when compared to the levels of these variables exhibited by LD 14:10 hamsters. Prolonged exposure to LD 6:18 resulted in a spontaneous increase in these three parameters to levels indistinguishable from those observed in LD 14:10 animals.

The onset of activity in all hamsters exposed to LD 14:10 occurred between 0-1 h after lights-off and did not vary appreciably for individual animals during the 210 days of LD 14:10. The time required for stable reentrainment following a shift from LD 14:10 to LD 6:18 varied between 30-120 days among individual hamsters and the phase relationship of the activity onset to lights-off after 200 days of LD 6:18 ranged between -1 and -6 h. The phase relationship of activity onset to lights-off was not affected by an increase in serum testosterone during spontaneous testicular recrudescence.

These results indicate that the number of wheel revolutions peaked and the lability onset of daily activity was controlled with photoperiod-induced changes in the hamster reproductive system. In contrast, the duration of the daily active phase and the phase relationship between lights-on and lights-off are relatively independent of changes in the reproductive system and are a function of the entraining light-dark cycle. (NIH HD-09885)
1503 EFFECT OF ELECTROLYTIC LESION OF THE MEDIAL RAPHE NUCLEUS ON 5-HTP + LUTI-171-INDUCED INCREASES IN SERUM PROLACTIN LEVELS IN RATS. Richard D. Flesser and Robert V. Melzer, University of Chicago Pritzker Schl. Medicine, Chicago, Ill. 60637.

Previous studies have demonstrated serotonin (5-HT) can stimulate prolactin secretion in the rat. In vitro studies have shown that 5-HT does not induce prolactin release by a direct effect on the pituitary. Several studies have suggested the median or doral raphe may be the locus of a 5-HT-induced release of a peptidergic prolactin releasing factor (PRF). To examine this question further, the effect of low dose 5-hydroxy-tryptophan (5-HTP) (30 mg/kg ip) plus 5-HT reuptake blockade with either L111 110140 (10 mg/kg ip) or Lu10-171 (5 mg/kg ip) was examined in rats which received either electrolytic lesion of the medial raphe nucleus (MR) or sham lesion. MR lesion one week prior to study significantly antagonized the serum prolactin increases in response to this drug regimen. In four separate replications, in these experiments, it was further demonstrated that: 1) neither 5-HTP, at this dose, nor administration of 5-HT reuptake blockers were positively correlated with significant increases in serum prolactin in either lesioned or sham animals, 2) the increased serum prolactin levels following 5-HTP + reuptake blockers were positively correlated with concentrations in the median eminence, 3) the increased serum prolactin concentrations were not dependent upon decreased concentrations of dopamine (DA) in the median eminence, and 4) total destruction of the MR was necessary to demonstrate total blockade of the 5-HTP + reuptake blockade-induced increases in serum prolactin. These results suggest that presynaptic 5-HT terminals located in the median eminence from cell bodies located in the MR, are necessary for serotonergically mediated effects on prolactin secretion induced by 5-HTP.

1504 EFFECTS OF DIFFERENTIAL FORNIX ABLATIONS ON THE CIRCADIAN RHYTHMICITY OF ADRENAL CORTICOSTEROIDS AND LOCOMOTOR ACTIVITY — A 48 HOUR STUDY. Christine F. Flachette, Henry M. Edinger, and Allan Siegel. Depts. of Physiology and Neuroscience, College of Medicine & Dentistry of New Jersey, Newark, NJ 07103.

In a previous study we showed that lateral fornix ablation in adult male rats disrupts the circadian rhythmicity of adrenal corticosteroids that is normally attuned to light-dark cycles, while medial fornix ablation alone and medial fornix lesion with white light also disrupted the rhythm. We hypothesized that the anterovenal subcircuit, the source of the medial corticohypothalamic tract (mcht), is responsible for modulating this rhythmicity function by nature of its link to the serotonergic and chiasmatic nucleus and the medial basal hypothalamus. In the present study we confirm and extend our previous findings by, 1) a pretraining on a period of observation to 48 hours, and 3) by examining the effect of differential fornix ablations on other rhythmic parameters, i.e., locomotor activity, body temperature, and blood and water intake. Seven (two to ten days after medial or lateral fornix ablation, animals were placed on activity platform. Blood samples were withdrawn at 4 intervals over a period of 48h by tail vein venipuncture in order that corticosteroid determinations could be obtained simultaneously with locomotor activity measurements. In addition, rectal body temperature.

Spectral analyis revealed that in 7 out of 10 lateral fornix ablated animals, the dominant period of the adrenal corticosterone rhythm (r) appeared at either 20 or 24h (r=20h24h). One of the animals exhibited a period of 30h, while the others demonstrated significant periods in the 40h range (r=40h25h). Further, in contrast, intact animals exhibited a period of 24-25h. In all animals, there were significant ultradian components. With regard to locomotor activity, all animals displayed dominant circadian rhythms regardless of corticosterone rhythms. The circadian rhythm of rectal body temperature was also disrupted in the lateral fornix ablated group. Our study supports the hypothesis of separate oscillators controlling circadian rhythms that are independent of the circadian oscillators.

(Supported by NIH Grant NS 07941-10)

1505 RESPONSES OF SERUM CORTICOSTERONE, FREE FATTY ACIDS, AND GLUCOSE TO FOOTSHOCK AND CONDITIONING STIMULI IN RATS. Michael J. Prey and Gary D. Cooper, Dept. Psych., Northern Illinois Univ., DeKalb, IL 60115.

Three experiments involving a session of intense electric footshocks delivered for 2 sec every 2 min were performed with male hooded rats. In the first two experiments elevations of serum corticosterone, free fatty acids (FFA), and glucose concentration were found within 10 min of the onset of the footshock session except under two conditions. That is, FFA did not increase significantly when the blood samples were obtained from the decapitated trunk of unanesthetized rats (Exp. 1). Also, the glucose elevation was not significant in nondeprived rats using unanesthetized decapitation, but was in 24-hr food-deprived rats (Exp. 2). In another experiment, serum prolactin increases in response to this drug regimen were found growing in the 3rd ventricle, juxtaposed to the anterior thalamus, and receiving a blood supply from the median eminence. Their median eminences were dissected out and either total destruction of the MR was necessary to demonstrate total blockade of the 5-HTP + reuptake blockade-induced increases in serum prolactin. These results suggest that presynaptic 5-HT terminals located in the median eminence from cell bodies located in the MR, are necessary for serotonergically mediated effects on prolactin secretion induced by 5-HTP.


The region of the hypothalamus containing the suprachiasmatic nucleus was dissected out of normal 17-day post coitum Wistar/Lewis rat embryos and stereotactically transplanted into the median eminence of adult female Brattleboro rats (which congenitally lack vasopressin-producing neurosecretory systems). The transplants were placed in the medial eminence of adult female Brattleboro rats (which congenitally lack vasopressin-producing neurosecretory systems). The transplants were placed in the median eminence of the lateral fornix ablated group we obtained a clear dissociation between dominant periods of locomotor activity and dominant periods of adrenal corticosterone rhythms. Lateral fornix ablation in adult male rats disrupts the circadian rhythmicity of adrenal corticosteroids that is normally attuned to light-dark cycles, while medial fornix ablation alone and medial fornix lesion with white light also disrupted the rhythm. We hypothesized that the anterovenal subcircuit, the source of the medial corticohypothalamic tract (mcht), is responsible for modulating this rhythmicity function by nature of its link to the serotonergic and chiasmatic nucleus and the medial basal hypothalamus. In the present study we confirm and extend our previous findings by, 1) a pretraining on a period of observation to 48 hours, and 3) by examining the effect of differential fornix ablations on other rhythmic parameters, i.e., locomotor activity, body temperature, and blood and water intake. Seven (two to ten days after medial or lateral fornix ablation, animals were placed on activity platform. Blood samples were withdrawn at 4 intervals over a period of 48h by tail vein venipuncture in order that corticosteroid determinations could be obtained simultaneously with locomotor activity measurements. In addition, rectal body temperature.

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(Supported by NIH Grant NS 07941-10)
3.1±2.9
137.3±22.1
(3)e
(3)
(9)
(3)e
0.87±0.17b
65.4±26.1
213.6± 9.4d
(9)
82.5±4.2c
(9)
(3)e
126.5±38.1
225.7±17.3d

Ovariec-tomized female rats treated systematically with estra-diol as a conjugate (E2) on 3 consecutive days reported an in-creased GAD activity in the substantia nigra (SN) and ventral teg-men-tal region (VTR) relative to oil treated controls. The aim of the present study was to examine the effect of local intracranial estrogen implants and the time course of the response following the estradiol(SEnt) experiment. First experiment, estradiol or cholesterol was implanted uni-laterally into one of five different brain areas. After three days of steroid exposure, the animals were sacrificed and GAD activity was measured in both the side of the brain in which the E2 was placed. Lastly, there was localization with regard to the SN and VTR, i.e., E2 Implanted in the SN induced a significant increase in GAD activity only in the VTR. In this second experiment, the time course of changes in GAD activity was measured in the SN and VTR after a single system injection of either 8 µg EB or oil. Rats were sacrificed at 0, 12, 29 or 53 h post injection. It was found that GAD activity in the SN was maximally enhanced at 29 h, whereas, decreased GAD activity in the VTR was apparent 12 h after EB had returned to normal by 29 h. Oil injections had no significant ef-fect on GAD activity. These results suggest that there may be two separate and distinct gangsteramic pathways which are differ-entially responsive to estrogen; a striato-nigral system projec-ting to the SN and a mesolimbic-VTR system possibly having cells of origin in the ACB. (Supported by NIH grants HD-01182 and GM07191 and the Ford Foundation)

Substance P (SP) has been found by immunocytochemical methods in fibers innervating a number of hypothalamic nuclei. These may be sites where this peptide acts to regulate the secretion of anterior pituitary hormones such as gonadotrophins, growth hormone and prolactin. Since we have recently demonstrated relatively dense projections of SP fibers to the magnocellular nuclei of the hypothalamus, particularly the paraventricular nucleus, central effects of substance P on the release of neurohypophysial hormones produced in these regions was studied.

Via chronic indwelling cannulae into a lateral cerebral ventricle (LCV), SP was administered to urethane anesthetized lactating rats and milk-ejection pressure was recorded as an indication of oxytocin release. Antidiuretic responses were followed after administration of concentrated sucrose solutions (23% w/w) to water-loaded rats. Injection of 1-4 μg (1-4 μl) produced a sustained increase of milk-ejection pressure in lactating rats and 0.2-0.4 μg produced long lasting antidiuresis in water loaded rats. 1 μg LCV β-endorphin blocked the milk-ejection response to SP, while the same dose caused profound potentiation of the antidiuresis induced by SP. Both the inhibitory and the potentiating effects of β-endorphin lasted at least 90 min. Although vasopressin is known to be an intrinsic milk-ejecting hormone, it is unlikely that its release accounts for this response in these experiments since β-endorphin had no effect on the antidiuretic or the milk-ejecting responses to substance P. If vasopressin accounted for the milk-ejection response then β-endorphin should have enhanced rather than inhibited the SP effect. The results also suggest that β-endorphin may participate in the regulation of differential secretion of oxytocin and vasopressin.

ELEVATIONS IN PLASMA CORTICOSTERONE IN RATS IN RESPONSE TO CONSUMPTION OF CONCENTRATED SUCROSE SOLUTIONS. Robert P. Hart*, Gary D. Cooper, Allan Shnerson*. Dept. of Psychology, Northern Illinois Univ., Dekalb, IL 60115, and William P. Smotherman, Dept. of Psychology, Oregon State Univ., Corvallis, OR 97331.

In Exp. 1, male hooded rats were given a 23% w/w sucrose solution or a choice between this sucrose solution and tap water after 36 hr of water deprivation.Sucrose consumption was completed within the first 15 min of the 120-min session, and was associated with a marked elevation in plasma corticosterone level (from 50 μg/100 ml within 60 min that remained elevated for the 120 min. The sucrose-water group consumed water after consuming sucrose, and exhibited a more rapid decline in corticosterone concentration during the interval between 60 and 120 min after the initiation of sucrose consumption. These data suggest that ingestion of concentrated sucrose solution facilitates pituitary-adrenal activity through the dehydrational consequences that continue after sucrose consumption is terminated unless subsequent water intake rehydrates the animal. As a test of this dehydrational hypothesis rats in Exp. 2 were deprived of water for 2, 24 or 48 hr and then consumed a sucrose solution (23% w/v). The three groups consumed the same amount of sucrose solution, and thus varied in degree of ultimate dehydration in accordance with hours of prior water deprivation. While all groups showed elevations in plasma levels of corticosterone, higher and more prolonged elevations occurred for the 24 than the 2, and for the 48 than the 24, hr deprived rats. As a further test of the dehydrational hypothesis, rats in Exp. 3 consumed one of six concentrations of sucrose solution (ranging from .20 to 1.166 mol/liter) on one of two concentrations of glucose solution (.470 or 1.166 mol/liter) after 36 hr water deprivation. Increasing the concentration of sucrose resulted in a decrease in amount consumed and an increase in plasma corticosterone concentration. Glucose consumption produced similar effects, though more weakly than equicaloric sucrose solutions. These data suggest that dehydration from consumption of highly concentrated sugar solutions inhibits further drinking and produces corticosterone elevations. The potentiation of rapid acute dehydration of corticosterone levels is surprising considering the minimal effects of water deprivation per se on corticosterone levels and the interpretation of pituitary-adrenal activation as a sign of psychological stress.


Sensitive GC/MS assays have been developed for quantification of picogram amounts of tryptamine (TA) and N,N-dimethyltryptamine (DMT). Using this method, TA and DMT were measured in single brains and adrenal glands taken from 120 day old male Sprague-Dawley rats. Known amounts of α, α, β, β-tetradeterated TA (DTA) and α, α, α, β-β-tetradeterated DMT (DDMT) were added to tissue samples prior to deproteinization and derivlization under acid conditions. The amines were extracted from the basified mixture with methylene chloride and derivatized with heptafluorobutyryl imidazole according to Benington, Christian and Morin (J. Chromatog. 106:435, 1975). The derivatized products of this reaction were taken up in 1 ml methylene chloride and washed with distilled water. Microliter portions of this mixture were found suitable for analysis of the heptafluorobutyryl derivatives of TA, DTA, DMT and DDMT. Determinations were carried out on a Hewlett-Packard 5985 GC/MS equipped with a Supelco 4' glass column containing 2% SP-2250 on 100-120 mesh Chromosorb W-NP. Quantification was obtained by a comparison of ratios of ion densities of standards specific for the heptafluorobutyryl derivatives of TA, DTA, DMT and DDMT from both pure standards and tissue extract.

These data indicate that DMT and TA are elevated in both brain and adrenals from animals that were individually housed for 28 days. If the animals are individually housed for 60 days, there is a decrease in the level of DMT from brain and adrenal while TA in these tissues remains elevated. This effect is enhanced considerably in younger animals. Further aspects of these phenomena are currently being investigated.

A very dense plexus of SP immunoreactive (SPI) fibers and terminals is present in the external layer of the median eminence (ME) in the human, monkey, domestic fowl and opossum, but not in the rat, guinea pig, mouse or hamster. The location of these SPI terminals is suggestive of a regulatory role for this peptide in anterior pituitary function. Antidenses raised in rabbits against synthetic SP were used in the indirect antobody PAP method to localize SP on 10μm coronal and sagittal sections of opossum brains that were fixed by intracardiac perfusion with Bouin's fixative. A SP fiber pathway was observed that projected to the ME. This fiber bundle begins to form in the medial preoptic area and courses caudally and ventrally adjacent to the III ventricle toward the ME. SPI perikarya were observed scattered among the beaded SPI fibers in the medial preoptic region. These observations suggest that the SPI perikarya in the preoptic area are the cells of origin of the SPI projection. In addition, SPI cell bodies were also seen in the arcuate nucleus. These cells may also contribute to the SPI eixeus observed in the ME. The specificity of immunostaining was established in control experiments in which anti-SP serum pretreated with an excess of SP failed to demonstrate the aforementioned structures on adjacent sections. We conclude that the SPI fibers and terminals in the ME of the opossum can be traced to the preoptic and the arcuate nucleus regions where SPI containing perikarya were observed. (Supported by the Snyder Fund and The Graduate School of The Ohio State University.)

Using an immunohistochemical technique (Cell Tiss. Res., 1975, 169:419-425) we localized a saturable binding material for melatonin (Mel) in the pineal gland (PG) of the rat. A quantitative double antibody procedure with anti-Mel (a-Mel) as the first antibody and biotinylated IgG as the second was used. Fluorescence intensity (FI) was measured with a Cadmium Sulphide photosensor.

PG from adult male rats (n=6) sacrificed 1.5 hr after lights-on showed successive increases in FI when 0, 10 ng, and 1000 ng Mel/μl were added to a-Mel before incubation. Adding 5 mg Mel/ml failed to increase FI above this level, showing saturation of the binding material. An estimate of the proportion of the binding material occupied by endogenous Mel, 0.30, was determined by dividing the FI for 0 Mel by the maximum FI from the saturation curve. Using this method, a diurnal change in the proportion of receptors occupied by endogenous Mel was found; the degree of binding increased progressively as the lighted portion of the day, reaching a maximum value of 1.0 shortly after lights-out. For this sampling point, the highest FI occurred when 0 Mel was added to the control which was incubated with antibody containing Mel and NAS (N-acetylserotonin). Thus, a daily rhythm of Mel binding in the PG exists, evidenced by changes in the proportion of the receptor population occupied by endogenous Mel.

Initial studies to determine the specificity of the binding element show that it binds both Mel and N-acetylserotonin (NAS). Sections of PG from rats (n=6) killed 1.5 hr after lights-on were incubated with antibody containing either Mel or NAS (1000 ng/ml). When an antibody which binds both Mel and NAS (a-MAS) was used, a 10-fold increase in FI was found when Mel was added, while only Mel increased FI. When a-Mel, which binds only Mel, was used as first antibody, only Mel increased FI.

In conclusion, this procedure provides a means of visualizing and quantifying Mel receptors in the PG. The application of this method to the study of Mel binding in other tissues will be discussed.

This work was supported in part by NIMH grant MH-14650.

1516 THE EFFECT OF ANTEROVENTRAL 3RD VENTRICLE REGION LESIONS ON OSMOTIC STIMULATION OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSAL SYSTEM. Alan Kim Johnson and Celissa D. Sladek. (SPON: Fred W. Mis). Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242, and Dept. of Physiology and Anatomy, Univ. of Rochester Sch. of Medicine, Rochester, NY 14623.

Rats with electrolytic lesions of the anteroventral 3rd ventricular (AV3V) region of the hypothalamus exhibit hypernatremia and chronic drinking deficits in response to hypertonic NaCl. These findings are suggestive of impaired osmoreception. The organ-cultured rat hypothalamo-neurohypophysal system (HNS) previously has been shown to release vasopressin (VP) in response to osmotic stimuli. The ventral portion of the region damaged by AV3V lesions is included in the HNS explant. Thus, these studies were initiated to evaluate the osmotic response of HNS explants which were obtained from rats previously prepared with AV3V lesions.

Following electrolytic ablation of the AV3V region or sham lesioning and a 2 wk recovery period, HNS explants were prepared and implanted into osmotic-sensitized or sham lesioned rats. The explants were maintained in organ culture for 4 days. On the third day in culture, increasing the osmolality of the culture medium from 295 to 315 mosm/kg H2O by the addition of NaCl resulted in a 2.5 fold increase in VP release from the sham lesioned explants. This increase was not significant in VP release from the AV3V lesioned explants. In the subsequent day in culture, acetylcholine (10-6M) stimulated a 3 fold increase in VP release from both sham and AV3V lesioned explants as well as the sham lesioned explants. These data suggest that the osmoreceptors which are involved in controlling VP release from the organ cultured HNS may be located in the region anterior to the ventral 3rd ventricle.

Supported by NIH grant AM-19761 and NIH Research Career Development Awards MH-00064 and NS-00259.


The purpose of the present study was to estimate the activity of tuberoinfundibular dopaminergic (DA) nerves during and after the elevation of serum concentrations of prolactin and luteinizing hormone (LH) which occur during proestrus, and which can be induced in ovariectomized rats by 0.5 mg/kg estradiol benzoate at 1000 hr; the control group received injections of corn oil vehicle. DOPA accumulation was determined at 800, 1200, 1800, 2400 hr on day 6 of 0.5 mg/kg estradiol benzoate at 1000 hr; the control group was sacrificed by decapitation. 

Prolactin, and possibly LH, appear to cause a delayed increase in the activity of tuberoinfundibular DA neurons, but that the elevations in the serum concentration of prolactin, and possibly LH, appear to cause a delayed increase in the activity of these nerves. (Supported by USPHS grant NS 9174.)

1518 IN VITRO EVIDENCE FOR MULTIPLE OSCILLATORS IN THE CHICK PINEAL GLAND. Charles A. Casale, R. Gin du Noall, Michael Henkater, J. Legino Perez-Polo. Dept. Zool., Univ. Texas, Austin, TX 78712 and Dept. Human Biological Chemistry and Genetica, Univ. of Texas Medical Branch, Galveston TX 77555.

In vitro studies done by us and others on chick (Gallus domesticus) pineal glands demonstrate that N-acetyltransferase, the enzyme involved in melatonin synthesis is under the control of a self sustained circadian oscillator located in the pineal gland. Cultured chick pineal glands maintained in constant darkness (DD) exhibit a circadian rhythm of NAT activity with peaks occurring approximately 24 hours apart and a phase which closely approximates that seen in vivo. These findings clearly suggest that the pineal gland contains one or more oscillators that can function independent of Innervation and external time cues.

In the present study we asked whether the organ integrity is essential for maintenance of this rhythm in culture. Chicks raised from one day of age in a light-dark cycle of 12 hours of light (LD 12:12) were sacrificed at three weeks of age and pineals were removed and placed into culture. Glands were bisected along the sagittal plane and each of the halves were placed into separate culture flasks. Respective halves were retrieved simultaneously during sampling. Examination of the first and second day in culture in DD revealed rhythmicity in NAT activity similar to that seen in whole-gland preparations with high levels occurring during the projected night of the birds prior light-dark cycle. Maintaining the organ culture in a continued LD cycle for two days and then releasing into DD again revealed a persistent rhythmicity in NAT during the third and fourth day in culture.

The persistence of rhythmicity in each section of the pineal gland demonstrated that the whole gland is not necessary for the production of a circadian rhythm and that the pineal gland contains at least two, if not more, self sustained oscillators which may function independently to produce a circadian rhythm. The issue of possible multiplicity of oscillators will be addressed in experiments entailing cultures of dissociated pineal cells.

Supported by NICHD grants HD15324 and HD14334, Robert Welch grant H698, and a RCDA (NS00213) to J.R.P.
1519 ANDROGENS CONCENTRATING CNS REGIONS OF THE SOUTH AFRICAN CLAWED FROG, *XENopus LaeVIS*: AUTORADIOGRAPHY WITH DIHYDROTESTOSTERONE. Darcy S. Kelley, Dept. Psychology, Princeton University, Princeton, N.J. 08544

Androgen concentrating regions in the CNS of 1, 5, 21, 28 and 35 days postnatal rats, ages 16, 17, 18, 20, and 21 days postcoitus (dpc) and 1, 4, 7, 14, 21, 28 and 35 days postnatal (dpm) were processed for formaldehyde-induced fluorescence of CA. All the CA sections that were taken for fluorescence were stained immunocytochemically, using anti-rat neuropein (RNP, provided by Dr. A. Robinson). RNP was visualized in the neuronal perikarya of SON and PVN on 17 and 18 dpc, respectively; the majority of immunoreactive cells stained very lightly, with a few cells staining darker. The numbers of darker staining perikarya, axons and dendrites increased with age in both nuclei, up to 28 dpm and remained essentially the same through 35 dpm. A few CA varicosities were also observed in the SON and PVN on 17 and 18 dpc, respectively. The density of CA varicosities in SON and PVN increased up to 14 dpm, beyond which there were only subtle differences among the remaining ages studied. The distribution of CA varicosities in SON and PVN began to resemble the adult pattern as early as 4 dpm, became more apparent on 7 dpm and was well established by 14 dpm with CA varicosities frequently encountered in close juxtaposition to the magnocellular neurosecretory neurons. The appearance of neurosecretory material in SON and PVN much earlier than the establishment of varicosity-cell contacts might indicate a trophic influence of the magnocellular neurons upon the ingrowing WE axons.

Supported by USPHS grants 5-T32-GM07230-04, AG00847, AG001456 and NSF grant BNS-78-11153.


Serotonin is known to affect ACTH secretion in mammals. The suprachiasmatic nucleus (SCN) of the hypothalamus is rich in serotonin and presumably modulates the rhythmic secretion of hormones including corticosterone (B). We studied the effect of SCN ablation or the administration of 5,7-dihydroxytryptamine (5,7-DHT), a serotonin neurotoxin, on diurnal B secretion. This was accomplished by either destroying the SCN bilaterally using a radio frequency lesion generator or injecting 150 μg of 5,7-DHT in 30 μl of an ascorbic acid-saline vehicle intraventricularly at diestrous in groups of 160 gm female Sprague Dawley rats. Animals given 5,7-DHT were pretreated with protriptyline (20 mg/kg, ip) to prevent uptake in norepinephrine neurons terminals. Data from the two experimental groups were compared to those obtained from sham-operated controls or following the intraventricular injection of the ascorbic acid-saline vehicle in protriptyline treated controls. Three weeks following neurosurgery, the rats were decapitated and trunk blood collected at 0700 hr (AM) and 1600 (PM) and B measured by radioimmunoassay. There was no significant difference in the mean plasma B concentration between those with SCN lesions compared with the 5,7-DHT treated group at either the AM or PM periods. There was no significant difference between the AM and PM mean plasma B levels in each experimental group. The actual AM and PM levels in both experimental groups were significantly higher (P < 0.05) and lower (P < 0.01) respectively, than the two control groups which had the normally expected diurnal difference in plasma B levels. We conclude that the effect of either SCN destruction or administration of a serotonin neurotoxin had a similar effect. These observations support the hypothesis that the SCN is involved in the regulation of the circadian secretion of corticosterone in the rat.

Ongoing studies indicate that the pineal gland secretes (both in vivo and in vitro) a non-indolic substance which inhibits LRF-induced release of pituitary LH. This pineal anti-LRF factor exhibits immunological characteristics which suggest that its structure may provide antigenic determinants similar to those of LRF itself; partially purified preparations of this factor cross-react with certain LRF antisera and both pinealocyte cell bodies and/or processes stain immunocytochemically with these antisera. A considerable number of analogs of LRF have been synthesized which are effective antiovulatory compounds; 2 of the most potent analogs are [3α,17β-Diol, 6 mg/100 g], [Ac-Pro¹, D-Phe², D-Trp³, D-Phe⁴]-LRF ("B-2") and [Ac-Pro¹, D-Phe², D-Trp³] -LRF ("K-43"). Antisera were generated against these analogs using a bisdiazotized benzidine conjugation to bovine serum albumin. Cross-reactivity with LRF was examined by RIA procedures and immunocytochemical characteristics were studied by applying the staining of median eminence and pineal gland. Antisera to analog K-43 recognized LRF and stained cells in the pineal gland; antisera to analog B-2 also stained cells in the pineal gland but did not recognize LRF. These results focus attention upon the N-terminal portion of the pineal anti-LRF substance with particular reference to positions 1 and 2.

Supported by grants from the National Science Foundation (No. NS-PC978-16123) and the Ford Foundation (No. 780-0616).


Immature female rats treated with 10 pg estradiol benzoate (EB) at 1200 h on d 23 and stimulated with 1 mg progesterone (P) 72 h later respond with a onanotropin (GT) surge (Calligaris et al., Endocrinol. 105: 97, 1977) which may lead to ovulation. Aromatizable, but not ring A-reduced androgens, can effectively be substituted for EB in this system (Kraull et al., Endocrinol. 105: 1822, 1978). As shown in the Table below, the ring A-reduced androgen, 5α-androstane-3α, 17β-diol (3α), 6 mg/100 g, can completely eliminate the estrogen-induced GT surge, if given 30 min before EB.

<table>
<thead>
<tr>
<th>EB only</th>
<th>EB/P</th>
<th>3α/EB/P</th>
<th>3β/EB/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (ng/ml)</td>
<td>58±29</td>
<td>1089±215</td>
<td>2116</td>
</tr>
<tr>
<td>FSH (mg/ml)</td>
<td>393±70</td>
<td>2178±409</td>
<td>3491±107</td>
</tr>
</tbody>
</table>

Implantation on d 21 of silastic capsules of the 3α-, but not the 3β-diol, led to a significant suppression in the response to ovulation (Kraull et al., Endocrinol. 105: 1822, 1978). As shown in the Table below, the ring A-reduced androgen, 5α-androstane-3α, 17β-diol (3α), 6 mg/100 g, can completely eliminate the estrogen-induced GT surge, if given 30 min before EB.

Supported by grants from the National Science Foundation (No. HS-PCH78-16123) and the Ford Foundation (No. 780-0616).


Since it is well documented for a large series of hormones (and also some neurotransmitters) that the response of a target tissue is largely dependent upon the level of specific tissue receptors, it becomes important to go into more detailed physiological characteristics of binding to the dopamine (DA) receptor. In order to investigate this specificity of pre- and postsynaptic receptors, the anterior pituitary, and the anterior pituitary membranes, the authors have selected DA agonists and DA antagonists as one of the most potent among them and DA agonists and DA antagonists as one target tissue of the anterior pituitary.

Supported by USPHS grants NS 06542, MH 25515 and 5 T05 CM 02220, and by Fellow Award 04-75/0167 to J. A. R. from the Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil.)
EFFECT OF SYSTEMIC MORPHINE ON AMYGDALOID SEROTONIN CONTENT AND SERUM LEVELS OF LUTEINIZING HORMONE. Joan M. Lakoski and Gerald F. McEwen, Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Ascending serotonergic projections to the amygdaloid nuclei and hypothalamic sites have been implicated in the regulation of the secretion of luteinizing hormone (LH). Recent evidence defines a role for these serotonergic pathways in the depression by opioids of LH release in the rat (Maughan et al., Brain Res. 130:41, 1978). To evaluate the role the amygdaloid nuclei might play in the depression of serum levels of LH, the effect of acute pararental administration of morphine (MOR) on levels of serotonin (5-HT) in 8 amygdaloid and l hypothalamic area was examined and correlated with MOR's effect on serum LH.

Male Sprague-Dawley derived rats maintained on a 12:12 light-dark cycle (lights on 0700 hr) were non-stressfully injected with MOR (20, 7.5, or 1 mg/kg), naloxone (NAL; 1 mg/kg), MOR plus NAL (7.5 mg/kg, NAL 1 mg/kg). Animals were decapitated 60 minutes after injection and the brain tissue was homogenized and stored at -80°C. Samples were punched from the following 8 amygdaloid nuclei (58 u diameter) and l hypothalamic area (508 u diameter) from frozen coronal brain sections and analyzed for 5-HT employing an enzymic-isotopic method (Saavedra et al., JPET 186:505, 1973): medial, cortical, basolateral, lateral posterior, central and the cortical, basolateral and lateral posterior caudal amygdaloic nuclei and the hypothalamic ventromedial nucleus (VMN). Serum LH was determined using a double antibody radioimmunoassay method (NIAMDD).

Control serum LH levels (20, 7.2 ± 4.9 ng/ml, n=15) were depressed by 60% in a dose-dependent manner. Serum 5-HT content in the amygdaloid nuclei and VMN after MOR administration did not significantly differ from control levels of serotonin at any of the MOR doses employed. These data suggest the amygdaloid nuclei may not be the primary site of opioid regulation of LH secretion by ascending serotonergic pathways. Supported by NIH grant NS 12114.

MELATONIN SPECIFICALLY INHIBITS PITUITARY RESPONSIVENESS TO LH-RELEASING HORMONE. Jeanne E. Martin and Carol Sattler.* Department of Pharmacology, Washington University Medical School, St. Louis, Missouri 63110.

Previous studies from this laboratory have shown that the pineal indole melatonin (MEL) can suppress the neonatal rat pituitary LH and FSH responses to LH-releasing hormone (LHRH). The present study has examined the specificity of MEL inhibition for the gonadotropins by measuring the effects of MEL on thyrotropin-releasing hormone (TRH) induced release of TSH and prolactin (PRL) and by using gonadectomized animals. In the absence of pulsatile cells in culture. Neonatal rat anterior pituitary cells were dissociated and cultured for 24h. The dispersed cells were washed with fresh medium and incubated for 3h in control medium or medium containing TRH (0.1-1000 nM) or LHRH (1-1000 nM) either alone or in the presence of MEL (10 or 1000 nM). Medium concentrations of TSH, PRL, GH, and LH were determined by double antibody radioimmunoassay with the use of materials supplied by the NIAMDD. As previously, MEL (10 nM) significantly (p<0.05) suppressed LH release by all concentrations of LHRH with inhibition of the response ranging from 17-56%. This concentration of the indole consistently produces maximal suppression of both LH and FSH responses to LHRH. By contrast, MEL at a 100-fold greater concentration (1000 nM) had no effect on either TSH or PRL release induced by any dose of TRH. In the absence of releasing hormones, medium LH increased only about 50% over zero-time values during the 3h incubation whereas the levels of TSH, PRL and GH increased 200-300%. While this apparent spontaneous secretion of TSH and GH was not affected by MEL (1000 nM), the indole has consistently induced a slight (16-18%), but significant (p<0.05) suppression of PRL release. These findings reveal that MEL inhibition of releasing hormone-induced pituitary secretion apparently is specific for LH. The data suggest, however, that MEL may also exert a regulatory effect on PRL secretion by the pituitary gland. (Supported by The Population Council).

Sprague-Dawley rats were implanted with chronic electrodes in the amygdala, preoptic area, and arcuate nucleus median eminence region in order to learn about electrical communication in the limbic system during the estrous cycle. Electrical signals were recorded simultaneously for ten minutes of each half-hour between 10:00 h and 15:30 h on each day of the estrous cycle. These signals were analyzed for amount and direction of signal traffic between pairs of sites. Signal traffic from the amygdala to the preoptic area lasts longer and takes longer on route during the "critical period" on the day of proestrus than at other times of the cycle. This is statistically significant at $p < 0.0002$ and implies a change in the signal pathway during this time. These data also imply that the arcuate-nucleus median eminence is probably not directly involved in initiating the release of gonadotropin-releasing hormone as a key event in the estrous cycle in the rat.

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<tbody>
<tr>
<td>AD(n=8)</td>
<td>229</td>
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<td>0.0128</td>
<td>3.17</td>
<td>0.0679</td>
<td>0.0180</td>
</tr>
<tr>
<td>AD(n=9)</td>
<td>220</td>
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<td>0.0117</td>
<td>3.18</td>
<td>0.0649</td>
<td>0.0181</td>
</tr>
<tr>
<td>N(n=9)</td>
<td>219</td>
<td>257</td>
<td>0.0189</td>
<td>3.51</td>
<td>0.0569</td>
<td>0.0190</td>
</tr>
<tr>
<td>NME(n=9)</td>
<td>219</td>
<td>284</td>
<td>0.0203</td>
<td>3.14</td>
<td>0.1230</td>
<td>0.0283</td>
</tr>
</tbody>
</table>

Comparison of the mean testes weights for the 4 groups revealed no significant differences. Pineal weights in groups AD and ADME were both significantly different from groups AD and NME ($p < 0.05$). In addition in groups AD and ADME were significant at the $p < 0.05$ level from groups NME and ADME. The only significant difference ($p < 0.05$) found in the thyroid weights was confined to the NME group. Group comparisons were determined by the Neuman-Keul analysis.


To examine alterations in lordosis behavior (measured by the lordosis quotient (LQ)) and dopaminergic function in female rats, we studied picrotoxin, a GABA receptor blocker, and hydrazino-propionic acid (HPA) which elevates tetrahydrobiopterin (TH) activity, as effects of these drugs on DA activity. Tyrosine hydroxylase (TH) and DA levels were measured. TH activity was found, but, Sham rats receiving HPA infusion had lower DA and HVA levels compared to those receiving saline, and SL rats receiving picrotoxin infusion had higher DA and HVA levels than those of saline controls. SL saline-infused rats showed decreased DA and HVA levels relative to Sham saline-infused animals. Thus, these three variables tend to be related in a particular way: when DA is low and GABA is high, the LQ is high, and conversely, when DA is high and GABA is low, the LQ is also low. These results support the concept of a GABA inhibitory neuronal feedback system which modulates DA turnover and perhaps plays a critical role in the neural control of lordosis behavior.

(Supported by NIH grants HD-01182 and GM07191 and the Ford Foundation)


In conjunction with an investigation of the pancreotropic effect of anterior hypophyseal extract (beef), we have initiated a study of the effects of this extract on other endocrine tissue with particular interest focused on the pinesal - testes axis. Male Sprague-Dawley rats (18), made insulin deficient with i.v. injection of 22 allantoic, 40 mg/kg body weight, were paired with 18 normal rats of comparable weight. Half of the allantoic-diabetic rats and half of the normal rats were injected (2 ml, s.c.) daily x 14 with a crude alkaline extract of beef anterior hypophyisis. On day 14 the animals were weighed, bled, anesthetized with Diabital (60 mg/kg) and perfused with 10% formalin for light microscopic observations or with 0.25% Karnovsky's fixative for EM observations. The pineal, testes, adrenals and thyroid were harvested from allantoic-diabetic (AD), allantoic-diabetic plus extract (ADME), normal (N) and normal plus extract (NME) groups. The initial body weight, final body weight, wet weight of the pineal, testes, adrenals and thyroid were recorded in gms. Statistical analysis of the numerical data was carried out with an electron desk computer (Sharp Comp III 364 P-II) using magnetic cards containing programs for multiple analysis of variance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body Wt.</th>
<th>Final Body Wt.</th>
<th>Body Fat</th>
<th>Weight</th>
<th>Testes Wt.</th>
<th>Adrenal Wt.</th>
<th>Thyroid Wt.</th>
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It has recently become apparent that opiate peptides interact with several neuroendocrine systems. In view of the similarities between the proposed control mechanisms of prolactin in mammals and melanocyte-stimulating hormone (MSH) in amphibians, we undertook a series of experiments to determine the effects of these drugs on DA activity. Tyrosine hydroxylase (TH) and DA and homovanillic acid (HVA) levels were measured. No effect on TH activity was found, but Sham rats receiving HPA infusion had lower DA and HVA levels compared to those receiving saline, and SL rats receiving picrotoxin infusion had higher DA and HVA levels than those of saline controls. SL saline-infused rats showed decreased DA and HVA levels relative to Sham saline-infused animals. Thus, these three variables tend to be related in a particular way: when DA is low and GABA is high, the LQ is high, and conversely, when DA is high and GABA is low, the LQ is also low. These results support the concept of a GABA inhibitory neuronal feedback system which modulates DA turnover and perhaps plays a critical role in the neural control of lordosis behavior.

(Supported by NIH grants HD-01182 and GM07191 and the Ford Foundation)
COINCIDENCE MODELS AND HAMSTER PHOTOPERIODISM. L. B. Morin
Dept. Psychol., Dartmouth College, Hanover, N.H. 03755.

The external coincidence model of photoperiodic time measurement predicts that the testes of male hamsters which have experienced protracted exposure to long days will always atrophy in the absence of photic stimulation. Three experiments were designed to test this prediction. Groups of reproductively mature male hamsters entrained to a long photoperiod were subjected to one of a variety of light cycle phase shifts followed several days later by blinding. Across all experiments, full gonadal atrophy occurred in 100% of the animals (N=58) not experiencing a direct shift or experiencing a phase advance, but failed to occur in 44% of animals (N=27) experiencing a 4 hr phase delay maintained for 10 days prior to blinding. A decrease in the period of the circadian locomotor activity rhythm was an after-effect of a phase advance or delay, respectively. Animals with atrophied testes showed relative compression of the gonadal atrophy occurred in 100% of the animals (N=58) not experiencing a direct shift or experiencing a phase advance, but failed to occur in 44% of animals (N=27) experiencing a 4 hr phase delay maintained for 10 days prior to blinding. A decrease in the period of the circadian locomotor activity rhythm was an after-effect of a phase advance or delay, respectively. Animals with atrophied testes showed relative compression of the gonadal atrophy occurred in 100% of the animals (N=58) not experiencing a direct shift or experiencing a phase advance, but failed to occur in 44% of animals (N=27) experiencing a 4 hr phase delay maintained for 10 days prior to blinding. A decrease in the period of the circadian locomotor activity rhythm was an after-effect of a phase advance or delay, respectively. Animals with atrophied testes showed relative compression of the gonadal atrophy occurred in 100% of the animals (N=58) not experiencing a direct shift or experiencing a phase advance, but failed to occur in 44% of animals (N=27) experiencing a 4 hr phase delay maintained for 10 days prior to blinding. A decrease in the period of the circadian locomotor activity rhythm was an after-effect of a phase advance or delay, respectively. Animals with atrophied testes showed relative compression of the
1540 DIFFERENTIAL CHANGES IN VASOPRESSIN IN SPECIFIC HYPOTHALAMIC NUCLEI AND CIRCUMVENTRICULAR ORGANS AFTER PROGRESSIVE DEHYDRATION. A. Negro-Vilar* and W. K. Samson* (SPON: J. M. Lipton). Dept. Physiol. Univ. of Tex. Bith. Sci.Ctr. at Dallas, TX 75235. Increased extracellular osmolality after water deprivation (WD) in the fetal hypothalamus leads to the osmolality of the hypothalamus. Taking advantage of a highly sensitive radiolmmunooassay for AVP combined with microdialysis techniques, we have analyzed the sequential changes in AVP level in several AVP rich areas such as the organum vasculosum laminae terminalis (OVLT) and in the periventricular nuclei (PVN) and in the serum of rats after 1-7 days of WD. Serum AVP increased progressively up to 3 days after WD, was still high by 5 days and declined to low levels by day 7 of WD. AVP in the PVN declined steadily to values below 10% of controls by day 7 of WD. AVP levels in paraventricular (PVN), suprachiasmatic (SCN) and arcuate (ARC) nuclei, declined slightly by 3 days and clearly fell below control levels by day 7. ME levels of AVP were also diminished after 7 days of WD. Levels of AVP in the SON remained unaltered throughout the 7 days of WD. AVP was also measured in the organum vasculosum laminae terminals (OVLT), paraventricular (PVN) and in the pineal gland. Changes in these circumventricular structures mirrored the changes in serum AVP, i.e. high values were seen at 3-5 days of WD and by 7 days levels had returned to low control values. To determine if the increased AVP content in the ME after 3-5 days of WD was associated with a change in the release of AVP from the ME, bilateral lesions were generated in this nucleus. Preliminary results suggest that SCN lesions reduced AVP release in the ME. Levels of AVP were lower than those measured in the SCN in normal rats. The results of the two experiments present a paradox. From the TSH results and the calculation of the T4:T3 ratios) that animals with hippocampal lesions were not significantly different from C and N animals in plasma TSH concentrations. The H animals had significantly more TSH than the C group and significantly less TSH than the T group. T6 (RIA) to T4 (RIA) ratios were calculated for each group. The H group was significantly lower than the C, N and I groups and significantly higher than the T group. The results of these experiments present a paradox. From the TSH results and the calculation of the T4:T3 ratios) that animals with hippocampal lesions were not significantly different from C and N animals in plasma TSH concentrations. The H animals had significantly more TSH than the C group and significantly less TSH than the T group. T6 (RIA) to T4 (RIA) ratios were calculated for each group. The H group was significantly lower than the C, N and I groups and significantly higher than the T group. 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The results of these experiments present a paradox. From the TSH results and the calculation of the T4:T3 ratios) that animals with hippocampal lesions were not significantly different from C and N animals in plasma TSH concentrations. The H animals had significantly more TSH than the C group and significantly less TSH than the T group. T6 (RIA) to T4 (RIA) ratios were calculated for each group. The H group was significantly lower than the C, N and I groups and significantly higher than the T group. The results of these experiments present a paradox. From the TSH results and the calculation of the T4:T3 ratios) that animals with hippocampal lesions were not significantly different from C and N animals in plasma TSH concentrations. The H animals had significantly more TSH than the C group and significantly less TSH than the T group. T6 (RIA) to T4 (RIA) ratios were calculated for each group.
EFFECTS OF PREOPTIC AND HYPOTHALAMIC STIMULATION ON SERUM LEVELS OF TESTOSTERONE AND CORTISOL IN INTACT AND GONADECTOMIZED MALE RHESUS MONKEYS ON TESTOSTERONE AND CORTISOL D. Collins*, Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30322.

Recent evidence has suggested that, in the rhesus monkey, social aggressive behavior may be correlated with fluctuations in gonadal and adrenal steroids. Electrical stimulation of specific regions of the hypothalamus and preoptic area can be effective for eliciting both aggression and the release of prolactin and gonadotrophin (luteinizing) hormone. The purpose of the present experiments was to investigate the effects of electrical stimulation of the preoptic area and medial basal hypothalamus on testosterone and cortisol as measured in RIA of serum samples obtained through chronically implanted venous catheters. A single set of stimulus parameters (0.4 mA, 1.0 msec, 50 Hz, 10 sec/min trains) was used for all subjects and all stimulus sites. These parameters had been determined in preliminary experiments to be suprathreshold for producing manifest behavioral responses. Stimulation was delivered unilaterally through a monopolar electrode arrangement for 60 min. Blood samples were taken 30 min prior to and just after the onset of stimulation. Samples were obtained at 15, 30 and 60 min following stimulation onset and at 30 min intervals for two hours post-stimulation. Positive hormonal responses were observed as 30-50% increases in serum concentration of cortisol or testosterone during stimulation over the mean of the two pre-stimulation values. Stimulation of the medial basal hypothalamus in both castrated and intact males produced increments as great as 2 to 5 fold in levels of both testosterone and cortisol. In castrated males the time course of change of both hormones was parallel. Increases in steroid levels in some instances remained elevated for more than 2 hours post-stimulation. Of the 40 tested sites that were histologically verified to be located in the preoptic area or hypothalamus, most effective sites were found in the POA (15%) and VMH (12.5%), with additional active sites located in LH and ANA. Negative sites, from which no response was obtained, were located in the MBH, DMH, SEPT and OC. Behavioral observations were conducted during stimulation and during the 60 min following; while the design was able to move freely in a situation. Some, but not all, situations from which testosterone and cortisol release could be elicited also supported stimulation-bound aggressive behaviors. The magnitude of the hormonal response to hypothalamic and/or preoptic stimulation was equivalent to or greater than the elevation in testosterone that occurred after episodes of social aggression. (Supported by NIH Grants NS 09688 and RR 00165.)


The region of the hypothalamus that includes the suprachiasmatic nucleus (SCN) is known to be responsible for the entrainment of endogenous circadian rhythm to the external light-dark cycle. It has been shown that the SCN receives an input from the retina, the ventral lateral geniculate nucleus, and the midbrain raphe, however the precise anatomical location and morphological characteristics of these neurons are unknown.

The afferent connections of the SCN of the female hamster were examined using the horseradish peroxidase (HRP) technique. A 301 solution of HRP in 0.05 M Tris (pH 7.6) was injected into selected regions of the SCN, and the brains were processed for light and electron microscopy. After a survival period of 24-72 hr, animals were perfused with 0.9% saline followed by 2.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The tissue was then postfixed with 1% osmium tetroxide, dehydrated, and embedded in Epon. Semi-thick (1-2 μm) and thin (0.2 μm) sections were cut and mounted on slides. The SCN was identified using the criteria of Fride and Irwin (1976) and studies of field and intracellular recordings in this species. The SCN was isolated and removed for histological analysis. The SCN was then dissected and the tissue was processed for light microscopy. The tissue was then mounted on slides and analyzed for the presence of HRP activity. The SCN was then dissected and the tissue was processed for light microscopy. The tissue was then mounted on slides and analyzed for the presence of HRP activity. The SCN was then dissected and the tissue was processed for light microscopy. The tissue was then mounted on slides and analyzed for the presence of HRP activity. The SCN was then dissected and the tissue was processed for light microscopy. The tissue was then mounted on slides and analyzed for the presence of HRP activity.

The ventral lateral geniculate nucleus (vLGv) was also labeled with HRP injected into the SCN. The rCB was also labeled by labeling ganglion cell terminals and transported to the retina. Labeled ganglion cells were large, relatively few in number and displayed no outstanding regional localization. After correcting for shrinkage resulting from fixation and histochemical procedures, the ganglion cell somata were not detected. In one retina, the time course of change of both hormones was parallel. Increases in steroid levels in some instances remained elevated for more than 2 hours post-stimulation. Of the 40 tested sites that were histologically verified to be located in the preoptic area or hypothalamus, most effective sites were found in the POA (15%) and VMH (12.5%), with additional active sites located in LH and ANA. Negative sites, from which no response was obtained, were located in the MBH, DMH, SEPT and OC. Behavioral observations were conducted during stimulation and during the 60 min following; while the design was able to move freely in a situation. Some, but not all, situations from which testosterone and cortisol release could be elicited also supported stimulation-bound aggressive behaviors. The magnitude of the hormonal response to hypothalamic and/or preoptic stimulation was equivalent to or greater than the elevation in testosterone that occurred after episodes of social aggression. (Supported by grants from the USPHS and Rockefeller Foundation.)
RELATIONSHIP OF DOPAMINE TURNOVER IN THE MEDIAN EMINENCE TO DOPAMINE CONCENTRATION IN HYPOTHALAMIC PORTAL PLASMA AND PRL ACTIVITY. \textit{H. H. N. Ph.D.}\textsuperscript{2}, J. P. Ph.D.\textsuperscript{2}, J. P. Ph.D.\textsuperscript{2}, and J. P. Ph.D.\textsuperscript{2}. \textit{Department of Biochemistry, University of Maryland, Baltimore, MD 21201.}

In the present study, we compared serum prolactin concentrations in rats on unpredictable and predictable stimulation. In contrast, predictable stimulation in rats resulted in a decrease in prolactin concentration. These findings suggest that decreases in prolactin concentration in rats on unpredictable stimulation are due to the release of dopamine from hypothalamic neurons. The release of dopamine from hypothalamic neurons was assessed by measuring the concentration of dopamine in hypophysial portal plasma and dopamine turnover in the median eminence. Dopamine was determined in acidified extracts of plasma or median eminence fragments using a radioenzymatic procedure.
Evidence from single unit recording and lesion-behavioral experiments has implicated the lateral medullary tegumentum in the mediation of estrous vocalization and arousal behaviors that precede genital stimulation in the female cat. The principal densities of estrogen-concentrating neurons in the cat brain, however, are in hypothalamic areas that do not make synaptic contact in the pineal. We decided to further elucidate the nature of the central neural network and the excitability of the pineal cells using conventional single and multiple-unit recording techniques. Stimulating electrodes of Wood's metal (40-60 μm tip) were placed in the pineal area of female rats. Extracellular AC recordings were made through glass-insulated tungsten electrodes (1-3μm, 1-10μm). In the pineal in situ, the spontaneous activity is of pinealocyte origin since neurons have not been identified in the pineal. Intracellular recordings will reveal the nature of this activity and whether it is coupled to the secretory function of the pinealocyte.

OKR was an Alexander Von Humboldt Fellow and MJK an NIH Fellow at the Max-Planck Institute.

Supported by NIH Grant NS-13748.


Recently we observed, using electron microscopy, degeneration of nerve terminals within the pineal for both habenula and inferior colliculus (Rønnekleiv & Wuttke, Exp.Brain Res., in press). It was earlier believed that these central fibers running from the habenula and posterior or commissural fibers do not make synaptic contact in the pineal. We decided to further elucidate the nature of the central neural network and the excitability of the pineal cells using conventional single and multiple-unit recording techniques. Stimulating electrodes of Wood's metal (40-60 μm tip) were placed in the pineal area of female rats. Extracellular AC recordings were made through glass-insulated tungsten electrodes (1-3μm, 1-10μm). In the pineal in situ, the spontaneous activity is of pinealocyte origin since neurons have not been identified in the pineal. Intracellular recordings will reveal the nature of this activity and whether it is coupled to the secretory function of the pinealocyte.

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LHRH is present in the OVLT and ME of the male (7.42 ± 3.30 pg/μg extracted protein, n = 10;25.06 ± 2.53 pg/μg, n = 9, respectively), ovariectomized females (1.01 ± 0.43 pg/μg, n = 11; 18.73 ± 4.37 pg/μg, n = 12), and random cycle female (3.06 ± 2.00 pg/μg, n = 40; 25.43 ± 3.04 pg/μg, n = 40) rats. In order to compare the physical properties of the LH-RH found in various structures of periphery, extracts from the OVLT, and ME were chromatographed on G-25 Sephadex (22 x 0.9 cm) by isocratic acetic acid 0.1M in distilled water. Fractions of 0.5 ml were collected. Aliquots of 0.5 ml of fractions were measured for LHRH content by RIA. In two separate experiments the elution profiles of LHRH were seen to be similar for OVLT and ME extracts. In 5 of the 6 rats and extracts from ME displayed a major peak of LHRH activity corresponding to fractions 1A and 1B. These peaks were found in 1000 hr and 1600 hr of the estrous cycle. The two main peaks of LHRH activity in OVLT were found in 1000 hr and 1600 hr of the estrous cycle. The two main peaks of LHRH activity in OVLT were found in 1000 hr and 1600 hr of the estrous cycle. The two main peaks of LHRH activity in OVLT were found in 1000 hr and 1600 hr of the estrous cycle.

The hippocampus has been widely used to study plasticity in the CNS. Previously we demonstrated that the sprouting in the dentate gyrus of the hippocampal formation could be altered with pharmacological doses of a glucocorticoid, hydrocortisone. In our present studies we have not chronicized physiological conditions which more closely approaches physiological conditions might also alter this sprouting response. Thus, we examined the response of the dentate gyrus of rats in which levels of glucocorticoids which more closely approaches physiological conditions are present following a unilateral lesion of the entorhinal cortex in control and hormone treated animals.

Young adult rats were adrenalectomized six to ten days prior to a subcutaneous implantation of a pellet containing a specified concentration of corticosterone utilized for chronic glucocorticoid replacement. Adrenalectomized and control animals were maintained on levels of corticosterone which more closely approaches physiological conditions that correspond to those in native young rats. In addition animals were maintained on levels which, were approximately twice those which are present in control animals.

Five days following implantation the animals were subjected to unilateral removal of the entorhinal cortex. The brains were examined for change in cortical input by means of AChE staining and for changes in the commissural-associational fiber plexus by means of the Holmes fiber stain. Control animals showed a sprouting response in agreement with previous results. It was found that animals maintained at the high levels of corticosterone showed significantly less sprouting than controls and animals maintained at low levels of hormone. Astrocytes appeared markedly hypertrophied in the high level group.

The present findings support our previous results with the administration of hydrocortisone. These findings suggest that steroid hormones regulate synaptic changes in the mammalian brain. Moreover, these results may provide a clue for understanding the mechanism underlying changes in synaptogenesis in aged animals. Previously we found that axon sprouting is reduced in aged rats and that glucocorticoids are elevated in these animals. The elevated glucocorticoids may be responsible for reduced sprouting in the aged animals. (Supported by research grant AG 00538)

DECREASED DOPAMINE TURNOVER IN THE MEDIAN EMINENCE IN RESPONSE TO SUCKLING IN THE LACTATING RAT. Michael K. Selmanoff and Phyllis H. Wise*. Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201.

The effects of suckling on the turnover of dopamine (DA) and norepinephrine (NE) were studied in terminal projection fields of the tuberoinfundibular (median eminence, ME) and nigrostriatal (caudate-putamen, CP) dopaminergic systems. Lactating rats with their litters were received between 1 and 5 days postpartum from the supplier. On day 9 postpartum the litters were culled to 8 pups and the dams were placed in indwelling right atrial cannulae under ether anesthesia. On day 10 postpartum pups were removed from their mothers for 4 hours and then returned. The onset of suckling was operationally defined as the time when 6 or more of the 8 pups started suckling. This occurred with a latency of 1-5 minutes from the time of pup removal. After 30 minutes of suckling free litters were obstructed to permit continuation of the stimulus from 30 to 60 minutes.

Catecholamine turnover was assessed by using the synthesis inhibitor α-methyl-para-tyrosine (αMPT). The drug was administered in acidic diluent (pH 5.2±0.5, 0.5±1.2ml volume) via the indwelling catheter (300mg/kg body weight) and the animals killed at the same time the pups were returned. In both situations the results were similar. Comparison of the rates of DA depletion after non-suckled and suckled dams showed a decrease in ME DA turnover in the suckled mothers. In contrast, dopamine turnover in the CN was not affected by suckling. Similarly, NE turnover in suckled mothers was not decreased while that in non-suckled mothers in the NE. These results are consistent with the hypothesis that dopamine is a physiological prolactin inhibitor factor mediating suckling-induced prolactin release. (Supported in part by NIH grants NS-14611, HD-02138 and HD-004355)

ESTROGEN MULLERIAN LOCUS REFRACTORYNESS TO PROGESTERONE IN SPONTANEOUS RATS. B.D. Berg, S.E. Harlan*, and R.R. Marks. Dept. Physiol., Univ. Texas Health Sci. Ctr., Dallas, TX 75235

Experiments were conducted to investigate the interaction between estrogen and progesterone in the display of feminine sexual behavior. Spayed rats received 5mL Silastic capsules containing 17α-estradiol (E,) and sc injections of progesterone (P;2.5mg) or ovariectomy (O) at 1200h, and were tested 18-2000h for lordotic responsiveness (LR) as measured by the lordosis quotient (LQ) in 20-sec tests (lights off: 13-2300h). Results are reported as mean ± SEM (N). A one way analysis of variance showed that in rats injected with E, to 27 h, 24 h after capsule injection (1200h) could not facilitate LR (at 18-2000h) P:32±10 (11); O:21±6 (10). In Experiment 1, E, capsules were removed 48 h after implantation at 1900h; P or O was injected at 1200h on this and the following day; and the rats were tested at 18-2000h on both days. Results indicated that while progesterone injected on P days 1-4 facilitated LR, E, capsules were refractory to a second P injection given 24 h after the first (O:36±9; 21±12 (11); P:88±6; 63±11 (11); P:0;9±5±73; 2±12 (9); O: 4±011; 5±4.12±10). In Experiment 2, E, capsules were left in vivo throughout the experiment. For O was injected at 1200h on the third and fourth days after implantation and the rats were tested at 18-2000h on both days. Results showed that a second P injection 24 h after the first can facilitate LR if E, capsules are present (O:0;47±14; 64±12±10); P:98±1; 98±10 (10); O: 96±16; 66±12±10 (10); P:59±12; 100±10 (9). In Experiment 3, E, capsules were left in viva during the entire experiment. For the first P injection at 17 or 20 h after the first P injection (given at 1200h). Results suggest that for the second P injection (given 24 h later) to facilitate LR, E, capsules must be removed for more than 1 h, with 11 h sufficing, following the first P injection or for longer than 52 h, with 62 h sufficing (second test: E, removed 3 h before P: 17±116; 6±11; 11±18±11±10 h after P: 17±116; 6±11; 10 h after P: 19±9±1; 9±1; not removed; 9±9±1) (6). Experiment 4 was conducted to distinguish between these two possibilities. E, capsules were left in vivo 62 h with the first P injection given 11 h before the first P injection. Results show that the presence of E, for 11 h, but not 1 h, following the first P injection facilitates the second P injection to facilitate LR (second test: E, removed 1 h after P: 19±16; 8±11; h after P: 9±7±1 (7). In summary, the results of the present experiments (a) reveal that 5mL Silastic capsules must be in vivo for more than 27 h for failure of the first P injection; (b) demonstrate the lordotic refractoriness developed in response to sequential P injections; and (c) establish that must be present longer than 1 h, with 11 h sufficing, following the first P injection for a second P injection given 24 h later to facilitate LR (Supported by HD 11814, HD 05885 and HD 05737).
1560 NORADRENERGIC CONTROL OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM. Celia D. Sladek and John R. Sladek, Jr. Dept. of Neurology and Anatomy, Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

In this wall of organ cultures of the hypothalamic nerve system, 1 mg of tissue were placed on a dish of medium and allowed to grow for 3 days. Following this period, the cultures were incubated with 5 nM noradrenaline (NA) or norepinephrine (NE) for 24 hours. The medium was then collected and analyzed for vasopressin (AVP) content. Results: Treatment with NA or NE led to a significant increase in AVP release compared to control cultures. These data indicate that the noradrenergic input to the organ culture system is inhibitory to AVP release. These findings may have implications for the regulation of neuroendocrine function and provide insights into the mechanisms by which noradrenergic afferents modulate the release of vasopressin.

1561 EVIDENCE FOR BRAIN HORMONE CONTROL OF LONG-DAY-INDUCED MATURATION IN A TERRESTRIAL MOLLUSC. Phillip G. Sokolove and J.B. Slone. Dept. of Physiology, University of California School of Medicine, San Francisco, CA 94143

178 male Long-Evans rats were stereotaxically implanted with either saline or a combination of ganglionic blockers to block autonomic nervous system outflow. Animals were subsequently exposed to long days of light (LD 16:8) and their reproductive tracts were examined 9-10 weeks later. These findings suggest that the noradrenergic input to the organ culture system is inhibitory to AVP release. These findings may have implications for the regulation of neuroendocrine function and provide insights into the mechanisms by which noradrenergic afferents modulate the release of vasopressin.

1559 DRINKING, VASOPRESSIN SECRETION, AND ACTH SECRETION INDUCED BY INTRACRANIAL ANGOTENSIN. J.B. Slone, M. Beed, L.C. Kell, T.R. Thrasher, and D.J. Ramsey. Dept. of Physiology, University of California, San Francisco, CA 94143

178 male Long-Evans rats were stereotaxically implanted with either saline or a combination of ganglionic blockers to block autonomic nervous system outflow. Animals were subsequently exposed to long days of light (LD 16:8) and their reproductive tracts were examined 9-10 weeks later. These findings suggest that the noradrenergic input to the organ culture system is inhibitory to AVP release. These findings may have implications for the regulation of neuroendocrine function and provide insights into the mechanisms by which noradrenergic afferents modulate the release of vasopressin.

With the PAP method two antigens in the same paraffin or vibratome section can be stained in consecutive reaction sequences, using diaminobenzidine as electron donor for peroxidase in the first sequence to obtain a brown color, and chloronaphthol in the second sequence to obtain a blue color. No removal of the immunoreagents is necessary between the two sequences even though anti-immunoglobulin and PAP are used in each. Anti-ACTH 1-24 and 1-39 stained fibers in the dorsomedial nucleus and fibers and perikarya in the arcuate nucleus. Fibers were distinct from those containing somatostatin, vasopressin and luteinizing hormone. Anti-ACTH 17-39 failed to stain these areas even though all anti-ACTH sera used stained cells in the pars intermedia (PI) and pars distalis (PD). Antiovine β-lipotropin (LPH, courtesy of Dr. C. H. Li) stained in the PI the same cells as the various anti-ACTHs, yielding mixed color reactions. Anti-LPH and anti-ACTH 1-19 stained in the magnocellular system and the internal zone of the median eminence the same cells and fibers as vasopressin, again giving mixed color reactions. Parabioses were either unstained or weakly stained with anti-LPH or anti-ACTH 1-39. No hypothalamic LPH reactivity was seen outside the magnocellular system. Anti-ACTH 17-39 and 1-24 failed to stain the magnocellular system. In the PD separate cells were stained brown and blue, when pairs of antisera to prolactin, growth hormone and ACTH were applied, except for a few cells that stained both for growth hormone and luteinizing hormone. Mixed colors were observed in the PI when pairs of anti-LPH and various anti-ACTHs were used in sequence. However when anti-ACTH 1-24 was followed by anti-LPH, separate cells stained brown and the basis for mixed color reactions the PD cell reactive with anti-LPH was identified as a glycoprotein-containing cell. The data suggest that in various parts of the brain may not always be identical to ACTH and that ACTH or β-LPH precursors are not necessarily coded by the same genes in different areas of brain and pituitary.


Work in our laboratory has indicated that catecholamine synthesis depends, in part, on the availability of the precursor amino acid, L-tyrosine (TYR). Since dopamine neurons play a major role in the inhibitory control by the hypothalamus of pituitary prolactin (PRL) release, we tested the effects of TYR administration on serum PRL levels. However, previous work has suggested that nigro-striatal dopamine neurons release more dopamine in response to TYR injection only after treatments that increase dopamine turnover. Therefore, we also tested the effects of TYR in chronically reserpinized rats. The injection of TYR (200 mg/kg ip) to male Sprague-Dawley rats had no effect on serum PRL levels. In contrast, TYR (200 mg/kg ip) significantly reduced serum PRL levels in chronically reserpinized rats (2.5 mg/kg twice daily for 4.5 days) (vehicle 138 ± 16 ng/ml; TYR 67 ± 7 ng/ml; p < .05) 1 hour after its injection. That this effect is mediated via increased hypothalamic dopaminergic release and decrease in hypothalamic levels of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC; +309%, p < .05) and homovanillic acid (HVA; +54%, p < .05) following TYR injection. TYR injection to non-reserpinized rats did not alter hypothalamic DOPAC or HVA levels. These studies demonstrate that a physiologic variable dependent upon catecholaminergic neurons can be affected by the availability of the catecholamine precursor, TYR, therefore, the inclusion of TYR may be a useful treatment for lowering chronically elevated PRL levels found in hyperprolactinemia.


In the present studies, estrogenized male rats with indwelling intraventricular cannulae were used. Catheters were implanted at least 3 days prior to testing and estriol valerate (1.25 mg/kg) was administered 3-4 days before each experiment. On the day of the experiment, extension catheters were attached to the indwelling cannula, the animals placed in individual cages, and a 45 min period of accommodation allowed. Muscimol (1.5 mg/kg) was adminis­tered i.v. to the conscious rats while blood samples were drawn into heparinized syringes at specified intervals prior to and following the drug. The samples were centrifuged and the plasma frozen for subsequent radiomimassay. Prolactin (PRL), growth hormone (GH) and TSH were measured by double antibody radioimmuno­assay. Plasma PRL response to muscimol described a biphasic curve. An initial elevation which peaked at 5-10 min was followed by a subsequent prolonged suppression at 30-60 min. The PRL ele­vations were blocked by increasing doses of glucose treatment, whereas the PRL suppression occurred even with haloperidol block­ade. GH secretion was not altered in the animal preparation either in whole hypothalamo-anesthetized animals. TSH briskly rose following muscimol in the conscious rat, however, was blocked by urethane anesthesia. GABA could alter pituitary hormone secretion through a direct effect of GABergic pathways on hypothalamic regulation of hormones or through an effect on another neurotransmitter possibly dopamine which regulates pituitary function.

Previously we have reported that the MPN, a small periventricular structure located immediately caudal to the organum vasculosum laminae terminalis is indispensable for the cyclic release of LH in the female rat. In the present study, effects of MPN lesions on (1) the estrogen (E2)-induced daily surge of LH and on (2) LH content in the medial basal hypothalamus (MBH) following injections of EB and P, were determined. Regular cyclic rats housed under standard lighting conditions were sacrificed 4-6 weekly following surgery. LH and MBH content of LH were measured by RIA. (1) Small electrolytic lesions were placed in 4 loci of the suprachiasmatic area. Lesions of the MPN and supra- chiasmatic nucleus (SCN) induced persistent estrus. Lesions in the locus anterior to the MPN (VPC) and lesions in the locus between MPN and SCN (ASR) were studied to determine their relationship to the plasma LH and P levels of LH and prolactin. Female Sprague-Dawley rats were ovarectomized 3-4 weeks previously and injected with 10 μg EB (day 0). On day 2 at 1000 half of the animals in each group were decapitated, tissue blocks from the MBH were removed for LH assay, and trunk blood was assayed for LH and PROLACTIN. Remaining animals received P on day 2 and were sacrificed at 1730. MBH content of LH in controls decreased from 1000 to 1730 (p<0.001) with a concomitant increase of serum LH. However, LH contents in both MPN and VPC lesioned groups were well below those of controls at 1000 and 1730 (p<0.001), despite the facts that MPN lesions abolished LH surge while the VPC-induced LH surge was blocked in all lesioned groups. After 1.5 mg P on day 3, a surge of LH comparable to that of sham controls was induced in VPC lesioned groups, but not in MPN lesioned groups. LH activity was increased 1 day after injection of EB (10 μg) and remained elevated on the second day. LH levels of LH and P, were determined in both EB- and P-induced LH surges in lesioned groups. LH activity was increased 1 day after injection of EB but remained elevated on the second day. The release of LH appeared to be inversely correlated with LH activity throughout the 3 day period unless no consistent correlation with prolactin levels could be demonstrated over the same time period. DBH activity remained unchanged except for a significant decrease on day 2 and day 3. The LH burst may be the key factor for the cyclic release of LH. (NIH RR00167)

INACTIVATION OF BRAIN CYTOSOL GLUCOCORTICOID RECEPTOR BY ATPase. Timothy J. Tyner, Michael Joy* and Richard V. Vardarson. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and Kent State University, Kent, Ohio 44240.

Previously we demonstrated the modulating effects of 100 μM concentrations of 17 beta-estradiol (E2) and testosterone (T) on in vitro slices of normal adult rats (Vardarson and Taylor, Neurosci. Abstr., 1978, A, 1145). In normal males and proestrus females E2 enhanced the amplitude of an extracellular population spike from the CA1 area of the hippocampal slice. In proestrus females the same effect was suggested by the presence of androgen and estrogen receptors in the embryonic brain. (Vito & Fox, Neurosci. Abstr., 1973, 3, A359; Science, 1979, 204, 517) or it may result from the priming effect of plasma gonadal steroids.

To investigate this question adult rats were castrated or ovariectomized prior to testing with 100 μM E2 and T. After allowing two days for plasma gonadal steroid levels to decline, hippocampal slices were prepared and tested to both E2 and T in an ABA design. Electrophysiological measures were taken at 10 and 20 minutes following addition of estradiol to the bathing ringer solution. CA excitation to monosynaptic stimulation of stratum oriens displayed the same properties as seen in the gonadally intact animals. That is, E2 enhanced the amplitude of the population spike in the castrated male. T enhanced the same response in the ovariectomized female. A tendency was noted for T to depress responsiveness in castrated males and for E2 to depress responsiveness in ovariectomized females.

These data are consistent with the hypothesis that hippocampal modulation by gonadal steroid hormones reflects sexual differentiation of hippocampal tissue rather than a response to circulating levels of hormone. The rapid onset of the steroid effect, within 10 min, may suggest a membrane effect of the steroid and is similar to the effect of E2 on hypothalamic LHRH neurons (Teyler et al., 1977, 35). These results indicate that steroid fluxes modulate the excitability of the rodent hippocampus and may do so by acting upon sexually differentiated neurons. (MSF grants BNS-77-28497 and BNS-78-23947 (TJT)).

MODULATION OF HIPPOCAMPAL EXCITABILITY IN ADULT CASTRATED AND OVARECTOMIZED RATS. Timothy J. Tyner, Michael Joy* and Richard V. Vardarson. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and Kent State University, Kent, Ohio 44240.

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1568 MODULATION OF HIPPOCAMPAL EXCITABILITY IN ADULT CASTRATED AND OVARECTOMIZED RATS. Timothy J. "Tyner, Michael Joy* and Richard V. Vardarson. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and Kent State University, Kent, Ohio 44240.

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These data are consistent with the hypothesis that hippocampal modulation by gonadal steroid hormones reflects sexual differentiation of hippocampal tissue rather than a response to circulating levels of hormone. The rapid onset of the steroid effect, within 10 min, may suggest a membrane effect of the steroid and is similar to the effect of E2 on hypothalamic LHRH neurons (Teyler et al., 1977, 35). These results indicate that steroid fluxes modulate the excitability of the rodent hippocampus and may do so by acting upon sexually differentiated neurons. (MSF grants BNS-77-28497 and BNS-78-23947 (TJT)).

Supported by NIH Grant HD 11922


Hypothalamic catecholamines have been postulated to play a role in the effects of estrogens on LH and prolactin release. In the present study, the effects of estradiol on the release of prolactin at 30°C in the presence of ATPase, the inactivation of glucocorticoid receptors by the two enzymes (Klopman, 1971, 4, A359; Science, 1979, 204, 517) or it may reflect sexual differentiation in the hippocampus of the same monosynaptic responses. This differential inactivation, whereas the steroid-bound receptor is unaffected. That is, E2 enhanced the amplitude of the population spike in the embryonic brain (Vito & Fox, Neurosci. Abstr., 1973, 3, A359; Science, 1979, 204, 517) or it may reflect sexual differentiation in the hippocampus.

Supported by NIH Grant HD 11922
Pituicytes have recently been implicated in the inhibition of neurosecretory axons. Gradually, these axons appear degenerative and are no longer present. In some cases, the axons are transiently wrapped in pituicyte cytoplasm, indicating their involvement in the surrounding tissue.

The activity rhythm of wheel-running in dark-pulsed hamsters showed splitting in 22 out of 22 hamsters. This splitting occurred either late in the subjective night or early in the subjective night, consistently altering the rhythm in three ways: 1) The rhythm phase was advanced by 0.5-2.0 h. 2) A new component of activity appeared in the phase dark pulse, and lasted for up to 5 cycles before returning to baseline. 3) The activity rhythm was split into two distinct components within a day after the pulse.

The present study indicates that dark pulses on a background of LL perturb the circadian system in a different manner than light pulses on a background of MD. Perturbation of the dark-pulsed rhythm may be a useful tool in examining the formal properties of circadian systems.

Corticosterone in brain and peripheral tissues was measured using radioimmunoassay, with 125I-labeled corticosterone as the tracer. The binding of corticosterone was highly selective and specific, with a high affinity for the corticosteroid binding sites in the brain. The binding of corticosterone in the baboon brain was found to be similar to that in the human brain, with a high density of binding sites in the hypothalamus and the pituitary gland.

In the primate brain, the predominant glucocorticoid is cortisol, with a B to F ratio of .75. The binding of cortisol in post-mortem brains (tissue concentration) was measured using a non-specific binding assay. The presence of cortisol in the brain was confirmed using a specific binding assay, which showed a high proportion of corticosterone binding in the brain.

In rats, the action of pituitary ACTH on the adrenal gland is well characterized. ACTH release is induced by various stresses, including surgical stress. In baboons, ACTH release is also induced by surgical stress, and the binding of cortisol in the brain was found to be similar to that in the human brain. The binding of cortisol in the baboon brain was found to be highly selective and specific, with a high affinity for the corticosteroid binding sites in the brain.
PUTATIVE ANDROGEN AND ESTROGEN RECEPTORS IN EMBRYONIC RAT HYPOTHALAMUS. Christine C. Vito, Sarah E. Bates*, and Thomas O. Fox. Dept of Neuroscience, Children’s Hospital Med. Ctr., and Dept. of Neuropathology, Harvard Medical School, Boston, MA 02115.

Sexual differentiation of brain occurs during a critical period of development which includes early neuronal and glial cell differentiation. The organizational influences of gonadal steroids upon a non-differentiated neural substrate occur during this period. We have investigated the distribution of adult-like estrogen receptors (Vito & Fox, Science 204:517-519, 1979) and androgen receptors (Fox, Vito & Wieland, Amer. Zool. 2001:18525-537, 1978) in the preoptic area, hypothalamus and telencephalon. Using standard receptor binding assays, we detected estrogen and androgen binding activities in all areas evaluated. The highest levels of estrogen receptors were detected in the ventromedial hypothalamus (VMH) and arcuate nucleus (ARC). These results confirm that estrogen receptor-like elements are present in the hypothalamus. Interestingly, the highest levels of androgen receptors were found in the VMH, lateral hypothalamus (LH), and preoptic area (POA). These findings suggest that androgens may play a role in the sexual differentiation of the hypothalamic regions that regulate reproductive behavior. The presence of adult-like estrogen receptors in the POA and VMH is consistent with the hypothesis that these structures are involved in the development of sexual behavior. The detection of androgen receptors in the VMH suggests that androgens may also be involved in the control of sexual behavior.

PITUITARY STALK SECTIONED RHESUS MONKEYS (MACACA MULATTA).

Embryonic and neonatal rat HOPOA also contain adult-like androgen and estrogen receptors. By biochemical techniques (DC) affinity chromatography, we detected macromolecular hormone-binding activities in cytosols of rat HOPOA as early as embryonic-day 15 (E15). Vaginal plug (VP) was set at E21.22. Androgen- and estrogen-binding activities in stalk sectioned monkeys, the results also suggest that this neurotransmitter, like dopamine, may mediate release of prolactin (PRL) by an effect on the pituitary. Within the same time interval. In both groups, PRL concentrations decreased within 10 min and reached baseline 50 - 60 min after the injection. These results confirm that serotonin releases prolactin in the normal rhesus monkey. Since serotonin induces a similar prolactin release in stalk sectioned monkeys, the results also suggest that this neurotransmitter, like dopamine, may mediate release of PRL by an effect on the pituitary.

Supported by NIH HD 10813 and SP30 HD 06132

The possibility that opioids play a role in the regulation of neurohypophysial peptide release is suggested by the presence of opioid receptors and endogenous fibers in the neurohypophysis (Nusser et al., Soc. of Neurosciences, Abstracts 4; 411, 1978), and the well-known antidiuretic action of morphine. Moreover, the morphine analogues, oxyclophlan (OXO) and butorphanol (BU) produce diuresis (Miller, Neuroendocrinology, 19: 247, 1978) and suppress the release of vasopressin which normally follows hypertonic stimulation (Banuolo et al., Fed. Proc. 37: 615, 1978). To investigate the type of opioid receptor mechanism possible involved, we studied the interaction of these analogues with the known potent opiate antagonist, Naloxone (NAL) on water diuresis. Six groups of adult male rats were used: a. saline (SA)+A. b. SA+OX. c. SA+BU. d. NAL+SA. e. NAL+OX. f. NAL+BU. Animals were kept in individual cages with water ad libitum. All drugs, dissolved in SA, or SA alone (0.2 ml./100 g) were injected subcutaneously. 1.0 mg/100 g. of NAL or saline was given at the beginning of the experiment (time 0), and 30m. later, 0.9 mg/100 g. of OX or BU was administered. Urine was collected every 30m. and volume and osmolality were measured. At the end of the experiment, the rats were killed by decapitation. Blood was collected in chilled tubes to measure plasma AVP levels. Rats receiving OX or BU developed a higher diuresis and produced urine with osmolalities significantly lower than the ones receiving SA alone or NAL plus SA. At the end of the third hour following drug administration urine and plasma osmolalities (mOsm/kg) for each group were as shown below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Urine</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OX</td>
<td>28d2</td>
<td>2542</td>
<td>2904</td>
</tr>
<tr>
<td>BU</td>
<td>28d3</td>
<td>2904</td>
<td>2853</td>
</tr>
</tbody>
</table>

These data confirm the diuretic effect of OX and BU, an effect not blocked by NAL which had no effect when given alone. Since the drugs BU and OX had effects opposite to that of morphine and not blocked by NAL they seem to be acting not as morphine agonists, but rather as antagonists. Moreover, they seem to be acting on antagonist receptors different from those with affinity for NAL. (OX and BU were kindly supplied by Bristol Lab. and NAL by Erco Lab.). Supported by NIH Grant NS No. 06624.


Urinary excretion following administration of naloxone, oxilorfan and butorphanol was studied in rats. Rats were divided into four groups: a. saline (SA)+SA. b. SA+OX. c. SA+BU. d. NAL+SA. Following injection of 0.9 mg/100 g b.w. of oxilorfan or butorphanol into male rats, a marked increase in urine output was observed. The increase was statistically significant compared to the control group. The highest increase in urine output was observed in the group receiving oxilorfan. The increase in urine output was not blocked by naloxone, indicating that the effect was not due to a direct action of naloxone.

Supported by NIH Grant NS No. 06624.

NEUROENDOCRINOLOGY


Retrograde axonal transport of horseradish peroxidase (HRP) or 125I-wheat germ agglutinin (WGA) was used to identify the cell bodies of origin of axons terminating in the median eminence (ME). The basal hypothalamus was exposed by a ventral approach through the soft palate and base of the skull. Pressure injections of HRP (10X, 0.02 to 0.04 μl) or WGA (1.9 x 10⁶ CPM/ml, 0.1 μl) were made under direct visual control into the ME of male and female rats through glass micropipettes (tip diameter, 6 to 10 μm). After 6 to 24 hours animals were sacrificed. Brains were processed for HRP with tetramethyl benzidine and for WGA by autoradiography.

Following injections into the ME, retrogradely labeled cells are found in the arcuate nucleus (ARC) and the periventricular region, as well as in the paraventricular nucleus (PVN), to a lesser degree, the supraoptic nucleus (SON) and other magnocellular neurosecretory cell groups. In the periventricular region, labeled cells are restricted to an area extending from the rostral part of the ARC to the level of the anterior commissure, and are particularly concentrated in the zone medial to the PVN. In the PVN labeled cells are found in all parts of the nucleus, but are most densely aggregated in the smaller-celled dorsomedial part. A few cells are also found in the rostral preoptic area lateral to the organum vasculosum of the lamina terminalis (OVLT). Cells of the ventromedial nucleus of the hypothalamus are not labeled in cases where the injection of WGA or HRP was confined to the ME, but are labeled if the injection spread into the ARC.

In order to determine if a portion of the labeling observed after ME injections was due to the spread of HRP into the posterior pituitary control injections were made directly into this structure. Following these experiments, the PVN, SON and all "accessory" magnocellular cell groups are very heavily labeled. Cells containing HRP reaction product are found in all parts of the PVN, but are most densely concentrated in the larger-celled anteroventricular and posterodorsalateral parts of the nucleus. In these areas virtually every cell is labeled. Scattered cells in the preoptic area lateral to the MEV and in the periventricular region near the ARC, and the preoptic region lateral to the MEV are not labeled after injection of HRP into the pituitary.
NEUROETHOLOGY
The cockroach is sufficiently sensitive that it detects and escapes from accelerations or weak accelerations. These "latency-clamp" stimuli, which can be more than 25 times larger than the just-threshold acceleration, while those at near-zero latencies activate an inhibitory (I) process (resulting in deceleration). The overall response is the sum of all BDC types and allows weak responses to drive E, while very strong responses would activate I. Another mechanism would be to pool responses from different differential sensitivity to different latencies: α-type cells respond to the inward current component of the EOD, β's to outward current, and δ's and ω's to both components. All of these responses may be amplified by foreign pulses at near-zero latencies with respect to the EOD. In addition, the δ-type cells and a subclass, ω', respond to foreign pulses at negative latencies.

A series of foreign pulses at fixed negative latencies evoke large EOD accelerations, while pulses at near-zero latencies result in decelerations of very small accelerations. If foreign pulses are briefly placed at a negative latency, then at a near-zero latency, a large acceleration results. Conversely, pulses at first a near-zero and then a negative latency result in decelerations or weak accelerations. These latency-clamp stimuli thus seem to mimic scanning situations, and furthermore suggest a theory for directional responses: pulses at negative latencies activate an excitatory (E) process (resulting in acceleration), while those at near-zero latencies activate an inhibitory (I) process (preventing response to the first, and/or causing deceleration; the process activated first then dominates the overall response.

There are two possible ways in which information from BDCs could be used to drive E. One way is to exploit the differential sensitivity to different latencies: α' and β types could activate E, while α, ω', and/or δ types activate I. Another pool response, all BDC types and allow weak responses to drive E, while very strong responses would activate I. The latter hypothesis is supported by experiments in which pulses at fixed latencies are amplitude-modulated: small vs. large modulations result in directional responses very similar to those obtained from scans and latency-clamps.
THE CENTRAL NERVOUS SYSTEM AND VOCALIZATION IN THE DOMESTIC CAT. 

The vocalization of the domestic cat are important in intra-specific communication. They are also expressions of emotion, understanding the odation of vocalization or brain lesion techniques can throw much light on these two aspects of species-typical behavior.

Studies by others on the domestic cat brain using either electrical brain stimulation or brain lesion techniques have implicated a number of brain regions in vocalization. However, the precise neuroanatomical pathways between these areas, or the impart to or output from these areas are unknown. The in which these areas influence vocalization have not also been investigated. With these questions in mind we have explored a region of the posterior fossa using electrical brain stimulation combined with silver staining of degeneration from sites placed at vocalization sites. Kanai & Wang (1962) and suggested that the above regions are sites on the call common pathway for vocalization in the cat. The existence of such a common pathway for all vocalization has been disputed by works on the Squirrel monkey (Jurgens & Plog, 1970). We found vocalization sites and lesioned by electrical stimulation. The threshold current for evoked-vocalizations ranged from 0.05 mA to 0.2 mA, and in the majority of cats was below 0.1 mA.

The vocalizations evoked from these sites were similar to naturally produced (a) soft meows, (b) low frequency (2 KHz) calls of long duration (grows), (c) calls typically very dense and evenly distributed representations of resonant frequency elements which extend up to 8 KHz (protest meows). His-like cells were evoked from these sites whereas purrs were never elicited. Little overt electroencephalographic activity accompanied evoked vocalizations. Typically, the cat would be seated on its belly, tail drawn forward, ears up and facing forward to lateral, with pupils narrow rather than dilated, non-aggressive vocal signs such as growls or defensive threat (e.g., locomotion, piloerection, ear-flatten -ing or arching of the back) were associated with these sites. Such behavior without vocalization was observed at sites dorsal to the call site.

Descending projections from the call sites were followed to the lateral pontine nuclei and the reticular formation. The facial, ambiguous and inferior olivary nuclei. Ascending projections were traced to the ipsilateral inferior colliculus, the nucleus of the thalamus, and the lateral lemniscus. It is suggested that the vocalizations evoked from these sites are the result of the activation of a vocalization control mechanism, and not the indirect result of stimulating pain pathways.

LEVELS OF FUNCTION IN RAT GROOMING BEHAVIOR. 

Decerebrate rats initiate spontaneous bouts of effective grooming. Thalamics, rats with more brain intact, are much less effective in their grooming (Grill and Norgren '78). Thalamicos perform grooming movements but do not correct directly these to their fur. Our hypothesis to define the components of grooming in intact rats and then to compare this normative data to that of chronic decerebrate and decerebrate rats. Male rats, 8 intact, 4 2-stage supragenual decerebrates (hand-held spatula transection) and 3 2-stage neodecerecates (aspiration) were used. Animals were observed in two conditions: spontaneous (no exogenous stimuli) and elicited (food, dirt on fur). grooming bout patterns were outlined; half of the observations were videotaped for slow motion sequential analysis. Intact grooming consisted of 21 components, including 5 types of face-washing, different grooming body areas, and 16 categories for grooming various body parts. Eye positions were determined by single frame videotape analysis.

When the tank is illuminated from the side a freely swimming fish can be oriented towards the light source (Dorsal Light Response,DLR). The amount of tilt depends on where visual and vestibular cues are matched. DLR is not evident when the photic stimulus is out (v.Holst,Z.vergl.Physiol. 22, 60 - 120, 1950). Bilaterally labyrinthectomised fish tilt their bodies to an angle which corresponds to the angle of maximum light intensity.

This behaviour is observed in normal, as well as in bilaterally labyrinthectomised fish. Although lesioned animals display more pronounced eye deviations than normal, the exact angle of tilt in lesioned fish never fully match the angle at which the light is directed towards them. Ocular deviations for these experiments are less than the physiological range of eye movements.

The data indicate that the DLR - model of v.Holst for bodily reactions cannot be employed for ocular responses. We conclude that mechanisms exist which control eye movements under the experimental circumstances described.
A quantitative ethological study of Sarsia medusae has been undertaken to establish what factors determine the timing of bouts of swimming in this animal. During the daytime, undisturbed Sarsia in aquaria alternate between periods of swimming by means of a series of bell contractions (swim bouts) and periods of quiet floating (pauses). Although the timing and duration of swim bouts and pauses can be very variable, the individual swimming contractions are initiated by an endogenous pacemaker. The behavior of Sarsia in aquaria was recorded over periods of six hours by a time-lapse videotape recorder. The videotapes were played back at normal speed and data were recorded onto a computer-compatible event recorder.

Three possible types of organization were considered in the analysis: 1) temporal organization, in which events occur with regular periodicity; 2) serial organization, in which the duration of an event is a good predictor of the duration of some following event; and 3) no organization, in which both of the first two types are absent. Fourier analysis was used to detect temporal organization. Serial auto- and cross-correlation coefficients out to lags of 23 were calculated and plotted as correlograms to detect serial organization. Three parameters were used in the correlation analysis: swim bout duration, pause duration, and interonset interval (the length of time between starts of swim bouts).

For animals that were swimming less than 25% of the time, there was a remarkable similarity in shape between swim bout and interonset interval autocorrelograms. This means that swim bout duration determined the interonset interval. In more active animals pause durations were more important in determining when swim bouts would start, perhaps because of fatigue. The pauses in active animals may represent a necessary period of recovery before the animal can swim again.

Fourier analysis showed that the bulk of the power spectrum is always concentrated at frequencies lower than .030 cps. Some animals showed a strong rhythm with a frequency of .005-.025 cps. All other animals had their greatest peaks at frequencies very near zero. A given animal usually had a characteristic spectrogram shape for up to three days. This suggests that the way swim bouts and pauses are coupled may vary somewhat from individual to individual.

Female sexual motivation can be expressed in distinct proceptive and receptive behaviors which function to initiate heterosexual courtship and facilitate mate acquisition attempts, respectively (Beach, Horm. Behav., 7, 105, 1976). For example, ultrasonic vocalizations by estrous female hamsters are attractive to males in the presence and absence of males, regardless of previous mating experience, but not during, male-female contact. In contrast, female lordosis clearly facilitates successful copulation and normally appears in response to tactile cues provided by a male just during close social contact.

The different roles played by hamster ultrasonics and lordosis in reproduction suggest that these behaviors depend on neural circuits that are at least partially distinct. To test this hypothesis, we studied the effects of proceptic area (POA) and ventromedial hypothalamic (VMN) lesions on female ultrasonic production and lordosis. Whereas destruction of either area was expected to affect lordosis (e.g., Nance et al., Brain Res. Bull., 2, 307, 1977), previous studies implicate the POA, but not the VMN, in the control of proceptive vocalizations (Nada et al., Horm. Behav., 9, 141, 1977).

Ultrasonic rates of ovariolectomized, hormone-primed female hamsters were observed in tests with stimulus males and ultrasonics. The postoperative call rates of females with bilateral electrolytic lesions of the POA or VMN were compared with preop rates for the same females, and with postop rates of females subjected to sham lesions. POA lesions consistently caused decreases in rates of ultrasonic production. In contrast, females with VMN lesions showed no consistent postop change in call rate.

Lordosis responses were observed in tests with stud males. Females with VMN lesions showed consistent decrements in lordosis. Females with POA lesions were more similar to sham-operated controls, but tended to show increased lordosis durations in postop tests.

These results show that brain lesions can dissociate the proceptive and receptive behaviors of female hamsters. In particular, VMN lesions affected receptivity (lordosis), but not proceptivity (ultrasound production). POA lesions tended to affect both proceptive and receptive behaviors, but in opposite directions. These results, then, emphasize differences between the brain mechanisms for proceptive and receptive components of female sexual behavior.

1595 COPULATION ELEVATES PLASMA β-ENDORPHIN IN THE MALE HAMSTER. Michael R. Murphy, Donald L. Bowie*, & Candace B. Pert. NIMH, Bethesda, MD 20205.

In men, use of opiate drugs can cause depressed libido, delayed ejaculation and impotence. In opiate addicts, withdrawal can cause increased libido, premature ejaculation and spontaneous erection. We investigate the role of endogenous opiate in sexual function by using a convenient animal model to (1) assay for changes in plasma β-endorphin during copulation, and (2) analyze the effects of opiate agonists and antagonists on mating behavior.

Male hamsters in one group were decapitated 30 sec. after their 5th ejaculation during the reflexatory period. Hamsters in a 2nd group were decapitated 30 sec. after a non-ejaculatory intromission following their 5th ejaculation. Members of a 3rd group were decapitated after removal from their home cage. Blood was collected in EDTA (5ml) and bacitracin (500μg/ml), plasma obtained and β-endorphin was extracted by alumina absorption. Radioimmunoassay of plasma extracts resulted in non-detectable levels of β-endorphin (0.00001-0.01M) in the unmated group. β-endorphin levels were at least 86 times higher (4.3x10-6M, p<0.01) in plasma collected following ejaculation. Levels in plasma collected after a non-ejaculatory intromission were slightly, but significantly, elevated (1.6x10-6M, p<0.01).

In other experiments the mating behavior of hamsters was tested after injections of saline; 1, 2, 4, 8, or 16mg/kg of methadone; 2, 20, or 200mg/kg of naltrexone; or a combination of both drugs. Sixteen behavior patterns seen during mating were recorded on a computerized event recorder and analyzed with respect to frequency, total duration, latency and average duration.

Methadone and naltrexone both caused a dose-dependent reduction in libido, with the methadone effect being the most severe. Potency (ability to achieve erection and intromission) was greatly reduced by methadone (2mg/kg) and naltrexone (20mg/kg). Pretreatment with naltrexone blocked the effects of methadone; posttreatment reversed the effects of methadone.

These results show that β-endorphin is elevated following ejaculation, during the post-ejaculatory refractory period, and could be involved in the inhibition of mating during this period. The behavioral results further suggest that endogenous opiates may be involved in the control of sexual potency and libido in the male hamster. Our findings confirm the hypothesis with recent reports that in rats opiate peptides inhibit the mating of sexually vigorous males (Meyerson & Tenerius; Gessa, et al.) and naltrexone induces mating in sexually inactive males (Gessa, et al.).


The giant Madagascar cockroach produces audible hisses in association with its courtship behavior. Two types of hisses, differing in amplitude modulation and temporal characteristics, occur in conjunction with 2 stereotyped behaviors performed by the male (Nelson, in press, J.C.P. & (1)). These observations raise several questions: are these hisses used as communicative signals, affecting the female's behavior? If so, what is the sensory basis of reception of such signals? How close is the reception matched to the acoustical characteristics of the sound?

All hisses produced by this species contain a broad range of frequencies extending into the ultrasonic, but most of the sound energy is concentrated in 2 broad peaks at about 4-8 and 12-16kHz. To determine the behavioral significance of courtship hissing, I first silenced males by blocking their external spiracles, and then restored the acoustical component of hissing by playing recorded courtship hisses to mute males and females paired with them at the appropriate moments of courtship. The timing and frequency of several courtship displays were compared across 4 groups of animals: normal, sham-operated, mute (silenced, no playback) and playback (silenced, sound playback provided).

The playback signal had a frequency cut-off of 16kHz.

Both muting and playback had significant effects on the outcome of courtship. Silenced males spent significantly more time in the early phases of courtship, but playback achieved significantly fewer copulations than males in control groups; the receptive behavior of females paired with them was significantly reduced from normal levels. In contrast, silenced males did receive playback, they achieved near-normal levels of copulation, and females paired with them showed normal levels of receptive behavior. Thus I conclude that acoustic information is a necessary feature of G. portentosa's social behavior.

To investigate the physiological basis of sound communication I recorded from the electrogenic complexes of minimally disturbed animals while playing both pure-tone and filtered noise stimuli. This revealed up to 4 interneurons which were driven by farfield sound stimulation, with the best frequencies ranging from 7 kHz. In the natural hiss this is the frequency range that contains the most energy. These preliminary data suggest that some neural elements involved in sound reception are tuned to the high-energy portion of the hiss's sound spectrum, in agreement with the observation that a playback signal lacking the higher-frequency components of the hiss was very effective in eliciting normal courtship behavior from females.


1597 THERE'S A CROWD: THE RESPONSES OF EIGENMANNIA TO MULTIPLE JAMMING ARE PREDICTABLE. Brian Partridge and Walter Heiligenberg. Scripps Institution of Oceanography, UCSD, La Jolla, CA 92037.

Most species of electric fish shift their electric organ pacemaker frequency away from potentially jamming signals, and a number of studies have investigated the behavior and its neuronal basis (e.g. Bullock et al., J.C.P. 77:1-48, 1972). With the exception of a brief mention by Bullöck et al., however, previous studies have examined the effect of only a single jamming stimulus whereas Eigenmannia normally encounters a number of jamming stimuli and must constantly be subjected to the electric discharges of several neighbors.

We have extended the model proposed by Heiligenberg and Bastian (abstr., this vol.) to predict Eigenmannia's response to multiple jamming stimuli. Close matching between predicted and observed responses provides independent confirmation of the model since it was originally developed to explain results from experiments with only single jamming stimuli. In the model, we represent the fish's EOD and jamming stimuli as vectors in the complex plane and draw resulting graphs of modulation of phase (H) and amplitude (S) of the combined signal in a 2-D state-plane. Shape of the graphs and their direction of rotation provide quantitative predictions for fishes' behavior during multiple jamming. Behavior of Eigenmannia in both intact and curarized conditions is well described by the model. These results demonstrate that fish also do not need to determine the separate DFs of jamming stimuli since attributes of the combined signal provide sufficient information for correct JARS.

Previous work has shown that Eigenmannia does not require an internal representation of its own EOD to produce correct JARS. Contrary to previous assumptions, our results demonstrate that fish also do not need to determine the separate DFs of jamming stimuli since attributes of the combined signal provide sufficient information for correct JARS.
DIRECT HEATING OF MOTHER RATS CURTAILS MATERNAL NEST BOUTS. Rodney J. Pelchat, Barbara Woodward*, and Michael Leon*. Dept. Psych., McMaster Univ., Hamilton, Ontario, Canada. Crosskerry, Smith and Leon (Nature, 273: 399, 1978) have suggested that thermal cues limit nest bout duration in rats. We tested this hypothesis by directly heating either the ventrum, core or precopital area of dams soon after nest bout initiation and measuring the time to nest bout termination. Ventral and core heating significantly reduced nest bout duration. However, termination of the bout was not concurrent with an acute, localized rise in temperature in either area but seemed to require time for temperature elevation in other areas as well. Diathermic heating of the precopical area terminated bouts very rapidly. Therefore, thermal factors are sufficient to terminate nest bouts. Continuous recording of maternal temperature also suggests that thermal factors play a greater role in nest bout termination late in lactation (Day 10 after parturition) than early in lactation (Day 0). These results, and control lesions produce only slight deficits. In unilaterally lesioned males, vision may play a role since some bilaterally lesioned animals cease burrowing once their eyes are beneath the substrate surface.

INHIBITION OF SHOCK-FACILITATED AGGRESSION IN FEMALE HAMSTERS BY SEPTAL STIMULATION. Michael Poteagal, Alan Blum*, Rosemary Beneny* and Murray Glueckman* Dept. Behavioral Physiology, N.Y. State Psychiatric Institute, New York, NY 10032.

In order to evaluate the full potency of septal stimulation to inhibit intraspecific aggression in the hamster (Poteagal et al., Soc. Neurosci., 4:364, 1978) we have modified our experimental procedure to maximize the frequency and intensity of aggression. We have found that septal stimulation will inhibit attacks by female Syrian golden hamsters (which are more aggressive than males in this species) even when these attacks are facilitated by foot shock. Our subjects were retired breeders which were ovariecctomized to eliminate the variability in aggressiveness accompanying the estrous cycle. Under our test conditions aggression levels of ovariecctomized females are not different from those of intact, non-estrous, animals (cf. Floody and Pfaff, JCPP 91:443, 1977). Subjects were then screened for high aggressiveness and implanted with bipolar septal electrodes. After recovery from implantation they were presented in their home cages with non-aggressive female target hamsters prepared in standard fashion (Poteagal et al., Soc. Neurosci., 4:364, 1978). Current values of 100 pps, 0.1 msec stimulation (82-370μA) that were sufficient to prevent the initiation of "spontaneous" (unshocked) aggression also proved sufficient to immediately stop already ongoing "spontaneous" aggression.

In subsequent daily sessions subjects were placed in a small shock chamber with a target animal and given ten 2 mA, 0.5 sec foot shocks at 30 sec intershock intervals (cf. Shipley & Kolb, JCPP 91:1056, 1977). Control sessions, C, (with no septal stim.) preceded experimental sessions, X, (with continuous septal stimulation at current values sufficient to inhibit "spontaneous" aggression) in the sequence: CXXXC. To date, subjects have attacked following 50% of C session shocks for a mean of 18.6 sec of attack/session; in X sessions subjects have attacked following only 7.5% of the shocks for a mean of 1 sec of attack/session.

Subsequent finch-jump tests of foot-shock pain thresholds show that septal stimulation at aggression-inhibiting current levels has had no effect to date on pain thresholds (mean control jump thresholds = 0.91 mA, mean stimulation-accompanied threshold = 0.90 mA). As in our earlier study, septal stimulation did not produce electrographic seizure activity in the septum and did not affect sunflower seed acceptance. It thus appears that septal stimulation is a potent and relatively specific inhibitor of intraspecific aggression in hamsters. (Supported by an H.F. Guggenheim Foundation Grant)
NEUROETHOLOGY

Aplysia brasiliiana swim by bilateral parapodial flapping with a rostro-caudal metachronal wave. Video tape records of freely swimming animals provide a noninvasive monitor of two components of this behavior, the period and metachronal offset. Changes in temperature affect the period (1.7 sec at 21°C to 3.1 sec at 12°C) but not the metachronal offset (0.5 sec at both temperatures). This results in a change in phase relationships between anterior and posterior oscillations at different temperatures and suggests that the period and metachronal wave have different underlying mechanisms.

Peripheral nerve lesions established that the anterior, middle and posterior parapodial nerves are necessary and sufficient for normal flapping with the anterior nerve playing the major role. Synchrony of flapping in the two parapodia is abolished by lesions of the pedal commissure, but bilateral removal of the pleural ganglia has no effect. Bilateral lesions of the cerebro-pedal connectives totally abolish swimming, but tonic stimulation of this pathway via a cuff electrode produces normal parapodial flapping for as long as the stimulus is maintained. Thus the command to swim originates in the cerebral ganglia but a neuronal oscillator for parapodial flapping is located within each pedal hemiganglion.

Phasic bursts of efferent activity in large units are recorded by implanted cuff electrodes during swimming in unrestrained animals. These bursts are synchronous in the anterior, middle and posterior nerves, despite the metachronal offset in the parapodial oscillations. Reducing the temperature produces a significant decrease in conduction velocity of large efferent units which will increase the offset in time of arrival of the efferent volley at the anterior and posterior parapodia. Therefore the lack of any effect of cooling on the metachronal offset suggests that neuronal conduction time distal to the cuff does not contribute substantially to this component. Phasic bursts of descending activity in large units are also recorded from the cerebro-pedal connectives during swimming.

Isolated brain studies support the hypothesis of a central pattern generator. Although difficult to trigger, phasic bursts of large efferent spikes alternating with bursts of smaller efferent spikes can be seen in some preparations, due to an overall reduction in number of contributing units. The increase in signal-to-noise ratio in isolated brains should make it possible to analyze single unit activity with existing spike separation techniques (Camp and Pinsker, 1979). (This research was supported by NSF grants BNS 76-17480 and 77-25584 to H.P.).
NEUROMUSCULAR JUNCTION
1603 REVERSIBLE ACCUMULATION OF ACETYLCHOLINE RECEPTORS DURING THE DEVELOPMENT OF AN AMPHIBIAN NEUROMUSCULAR JUNCTION IN CELL CULTURE. M. J. Anderson* and F. G. Kilt* (SPON. A. Selverston) Salk Institute, San Diego, CA. 92120.

Cells from the myotomal muscle and neural tube of the embryos of Xenopus laevis were grown together in culture. Over a period of 7-21 days, clusters of cells to established muscle cultures, discrete acetylcholine receptor accumulations could be detected along the path of nerve-muscle contact after staining with tetramethylrhodamine-labelled α-bungarotoxin. Sequential observations on individual muscle cells over a period of 16-20 hours revealed a progressive formation and growth of discrete acetylcholine receptor aggregates at sites of nerve-muscle contact. Examination of identified nerve-contacted cells in the electron microscope further revealed that the larger regions of receptor accumulation corresponded to sites of extensive synaptic differentiation. These regions of cell-contain clusters of synaptic vesicles, post-synaptic membrane thickening and an intercellular specialization of the basal lamina. When such cells became denervated, either spontaneously or as a result of severing the developing neurite, the dense receptor accumulations along the nerve decreased in area or disappeared entirely within 16-20 hours. On the basis of these observations it is concluded that aggregates of functional acetylcholine receptors remain dependant upon some continuing action of the nerve at these early stages of synaptogenesis.


At the frog neuromuscular junction a double logarithmic plot of evoked transmitter release vs. extracellular (Ca) has a steep, nonlinear dependence of Ca on release. However, Crawford (1974) reported that when evoked release rates are lowered still further, the relationship between release and extracellular (Ca) changes rather suddenly from fourth power to linear. We reexamined the Ca dependence of evoked transmitter release at very low release rates using surface neuromuscular junctions of the frog cutaneous pectoralis muscle. The average quantal content (m) of the end-plate potential was reduced to very low levels (between 0.002 and 1) by reducing bath [Ca] and adding 2 mM Mn, 4 mM Co or 10 mM Mg. We found that when the motor nerve was stimulated at low frequencies (0.5 - 2 Hz) in Mn or Co, m was proportional to the fourth power of extracellular [Ca?] down to the lowest m values we could measure, 0.002 to 0.003. Together with Dodge and Rahamimoff's earlier results, this result suggests that some step in the transmitter release pathway has a steep, nonlinear dependence on bath [Ca?] over more than three orders of magnitude of release rates. When 10 mM Mg was added to the bath, the lowest m values were markedly higher than the fourth power prediction. Increasing the stimulus frequency to 5 - 20 Hz progressively increased both the m and the rate of 'background' release during the interstimulus interval. In the range of low quantal contents studied here, frequency enhancement of both evoked and background release was more pronounced in 10 mM Mg or 4 mM Co than in 2 mM Mn. These results suggest that Mg and possibly Co are weak activators of transmitter release, so weak that their activating abilities are evident only when normal Ca-activated release is greatly reduced. This frequency-dependent enhancement of low release rates in Mg and Co probably accounts for the difference between our and Crawford's results. Supported by NIH grant NS 12004.


Medium conditioned by contact with NG108-15 clonal neuroblastoma x glioma hybrid cells contains a factor which increases the number of acetylcholine receptor (ACR) aggregates on cultured myotubes (Christian et al. 1978, PRS 75: 4011-4015). A factor with this activity is also found in the cytoplasmic fraction of the hybrid and cells in the cytoplasmic fraction of embryonic rat brain. ACR aggregation activity was not found in the cytoplasmic fraction of adult rat brain. The ACR aggregation activity of hybrid cells conditioned medium or the cytoplasmic fraction of hybrid cells was concentrated by ultracentrifugation, loaded on a Sephacyr 5-300 column and eluted in 50 mM Tris HCl buffer as a fraction with a molecular weight of from 150,000 to 200,000 daltons. At neutral pH, 80% of the protein in this active fraction bound to a DEAE cellulose column, and ACR aggregation activity was eluted in a linear NaCl gradient at an approximate salt concentration of 300 mM. Further separation on a Sephacyr 5-300 column resulted in detectable aggregation activity at 4 μg of protein per ml, indicating purification of the starting material of over 10 fold. In addition, a factor which depressed the number of ACR aggregates on cultured myotubes was separated from the aggregation activity. Part of the receptor aggregation activity in conditioned medium fraction to concanavalin-A-Sepharose and was eluted by α-methylmannoside. Hence, a neuronal factor which modulates ACR aggregation appears to be a large, negatively charged glycoprotein.


1) In muscles totally denervated at birth, few or no additional muscle fibers were produced. 2) In muscles with only 8 remaining motor units, the total number of muscle fibers in muscles with different numbers of remaining motor units. The results showed the following: 1) In muscles totally denervated at birth, few or no additional muscle fibers were produced. 2) In muscles with only 8 remaining motor units (normal is about 12) mature muscles contained the normal adult number of muscle fibers. 3) The overall relationship between the number of muscle fibers and the number of remaining motor units could be described with reasonable quantitative accuracy by a simple model.

The syrinx is the organ of song production in passerine birds and is innervated by the branch of the XII cranial nerve. In many passerines, song is an important component of male sexual behavior, and it disappears following castration.

Recent works have shown that the muscles and the motor neurons which contain them transport receptor for testosterone (T) which are specific and high affinity (Arndt et al., J. Comp. Neurology 165:467, 1976; Lieberburg & Nottebohm, J. Exp. Zool. 188: 294, 1979). The additivity observed with HTX and 2-propanol (kdis = 4.3 × 10^7 M^-1 s^-1) and partially restored ChAc (3299 ± 176). A similar pattern was observed for AChE and cholinergic activities in song control muscle.

In this study, we examined the effects of altered circulating T levels on the tracheosyringeal nerve by measuring nerve choline acetylase (ChAc) and AChE. We have further explored the effects of T on song muscles AChE using sucrose density gradient analysis. Adult male ZFs were castrated and implanted one week later with Silastic capsules containing cholesterol (C) or T. Four to 5 weeks after castration, nerve ChAc levels declined by 34% (from 3678 ± 229 umoles/gm protein/hr. in intacts to 2444 ± 434 in T treated castrates). AChE levels decreased by 60% (187 ± 207 umoles/gm protein/hr. to 73 ± 93). T replaced testosteron shows the same activity restorable (3) and partially restored ChAc (3299 ± 176). A similar pattern was observed for AChE and ChAc activities in syringeal muscles.

The effects of T on activities in other vertebrates is an important area of research which is not well understood. In this context, we have measured the activity of AChE in the absence and presence of ACh. In the absence of ACh, the L peak predominated strikingly, whereas the activities in the H and L peaks were more similar in the larynx and trachea. Syringeal AChE was analyzed in intact birds and in castrates treated with T or C in Silastic capsules, as above. In contrast to the marked effects of T depletion on both total and specific AChE, no changes in the distribution of AChE activity between the three peaks was observed after castration (Supported by PHS Grants HD12011 and MH18343, and by RF009 from the Rockefeller Foundation.)


The equivalence between the presence of large numbers of 11 - 19 nm IMPs observed during the developmental period in vivo and in vitro. ACh sensitivity in frog embryos occurs between Nieuwkoop and Faber stages 19 and 24. Embryos from which intracellular recordings had been taken were freeze-fractured. Preliminary results from these identified 11 - 19 nm IMPs. We tentatively conclude that ACh sensitivity may occur almost simultaneously with the appearance of ACh sensitivity. This lends further support to the proposition of 11 - 19 nm IMPs as a possible form of ACh sensitivity. (Supported by USPHS Grants NS 10457-06; NS 08601-10 and 5-T32-ES07212.)


The nicotinic cholinergic receptor in postsynaptic membranes isolated from Torpedo electric tissue has been shown to exist in two interconvertible conformations, one binding acetylcholine (AcCh) with low affinity and associated with channel activation (RcAcCh) and a second binding ACh with high affinity (RHiAcCh). The RHiAcCh state of the receptor is thought to represent a conformation of the receptor with excitatory amino acids.

In order to determine the mechanism by which noncompetitive antagonists cause receptor desensitization, we have measured their effects on receptor conformational equilibria. In the absence and presence of ACh, we observed three peaks of AChE activity in the absence of ACh, the L peak predominated strikingly, whereas the activities in the H and L peaks were more similar in the larynx and trachea. Syringeal AChE was analyzed in intact birds and in castrates treated with T or C in Silastic capsules, as above. In contrast to the marked effects of T depletion on both total and specific AChE, no changes in the distribution of AChE activity between the three peaks was observed after castration (Supported by PHS Grants HD12011 and MH18343, and by RF009 from the Rockefeller Foundation.)

1610 THE EFFECTS OF Ca ++ DEPRIVATION ON ACCUMULATIONS OF ACETYLCHOLINE RECEPTOR IN THE DEVELOPING NEUROMUSCULAR JUNCTION. Robert J. Bloch* and Joe Henner. Steinbach Neurobiology Laboratory, The Salk Institute, San Diego, CA. 92121.

One of the earliest events in the formation of the neuromuscular junction is the accumulation of a high density of acetylcholine receptors (AChRs) in the synaptic membrane. In order to determine what forces are involved in maintaining and maturing these primitive synaptic structures, we have subjected fixed frog embryonic muscle fibers to different stages of development to disruptive treatments, and, in particular, removal of Ca ++ . Sternomastoid muscles were excised and pinned out on plastic dishes. ACh was visualized using a tetramethyl-rhodamine derivative of α-bungarotoxin (R-αBt). Removal of Ca ++ was accomplished either by culturing in Dulbecco's-modified Eagle's medium prepared free of Ca ++ or by using medium buffered with the Ca ++ chelator, EGTA. When muscles from 16 day embryonic rats were treated for 6 hours at Ca ++ -free medium containing decreased concentrations of free Ca ++ (<200 μM), extensive loss of AChR accumulations was observed. This loss was independent of whether staining with R-αBt was performed before or after incubation in Ca ++ depleted medium. Muscle fibers treated with Ca ++ -free medium decreased levels of protein synthesis. Physically damaging muscles did not cause loss of AChR accumulations. Thus, Ca ++ deprivation, while it disperses AChR accumulations at the newly formed neuromuscular junction, does not appear to do so by damaging muscle. As a function of developmental time, junctional AChR accumulations become increasingly resistant to the effects of Ca ++ deprivation. This was already observable with neonatal sternomastoid muscle and was complete by 3 weeks after birth. Our results suggest that AChR accumulations at the developing neuromuscular junction are less stable than accumulations at mature junctions, and that stabilization occurs relatively rapidly after formation of the junction.

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COATED VELOCICLES IN CULTURED MYOTUBES CONTAIN ACETYLCHOLINE RECEPTORS. S. Brerett and G.D. Fleisch, Dept of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

Chick myotubes and spinal cord explants were cocultured on glass coverslips. After myoblasts had fused to form myotubes, spinal cord slices cut from 12 day ciliary ganglia were added to the cocultures. For electrophysiology the EDII was removed and continuously superfused with oxygenated Locke's solution. Intracellular recordings showed that part of this decrease reflected the elimination of synapses from polyneuronally innervated muscle fibers. In several instances coated vesicles opened on the cell surface and contributed to the postnatal density. In some postsynaptic membrane density was observed to be clustered in the opposed nerve process. Coated vesicles did not appear to fill with or bud from the synapses. The possibility that coated vesicles contain acetylcholine (ACh) receptors was investigated with HRP-α-BTX conjugates, after the cells membranes were permeabilized with 3-mercaptoethanol buffered rinses followed by a 3% saponin wash and at 21% deg. Muscle fiber staining potentials (HRP-α-Btx) were recognized intracellularly, electrodes placed close to endplates located using Hoffman modulation contrast optics. At all ages examined HRP frequency was lower at dystrophic than at normal endplates. In dystrophic muscle fiber membranes time constants (HRP-α-Btx) were recognized intracellularly. E-PTA also stains a prominent band beneath the submembranous density and separated from it by a space. The specialized membrane area is closely associated with small clear synaptic sites of myogenic cells. At sites of early nerve-muscle contact, patches are more numerous and larger. Often the external coating extends a greater distance into the extracellular space forming bridges with a sparse coating on the surface of growing nerve fibers. In more highly developed junctions, the external layer and submembranous density occupy the entire postsynaptic region. This study reveals the presence of external and internal specializations at regions of high ACh density and at sites of initial nerve-muscle contact. These specializations may be involved in the localization of positions of AChR in the membrane or contain components which establish these areas as preferential sites of nerve contact. (Supported by the Osserman Fellowship of the Myasthenia Gravis Foundation and NIGER BRS 78-13729.)

MORPHOLOGY AND ELECTROPHYSIOLOGY OF DYSTROPHIC CHICKEN MUSCLE. Joan S. Bryan* and Michael S. Letinsky. Dept. Physiol., Sch. Med. U. of Cal., Davis). These are new dystrophic and control lines (EDII) have been examined histo-lo-gically (7 wks) including: extensive variation in muscle fiber diameter; distance in muscle fiber diameter, muscle fiber hypertrophy, increased number of muscle fibres; cisternae are centrally located, muscle fiber splitting and increased muscle connective tissue.

Magnification revealed that there was a 5.5 fold increase in the size of the motor unit during postnatal development of lumbar muscles. J.H. Caldwell*, W.J. Betz, and R.R. Ribchester* (SPON: A.R. Martin), Dept. Physiol., Univ. Colorado Med. Sch., Denver, CO 80262.

The number of muscle fibers innervated by individual motor neurons (motor unit size) was measured in lumbar muscles of rats aged 0-28 days, during the period of elimination of polyneuronal innervation. Motor unit sizes were determined from twitch tension measurements combined with muscle fiber counts. Tetanic tensions contributed by individual motor units declined from about 25% of the twitch tension at birth, to about 9% at 28 days. The number of units increased with age in dystrophic muscles, it was still only 72% of control frequency (5.4 per min); and although Mepp frequency increased with age, it was still only 72% of control by 13 wks. The possibility that coated vesicles contain acetylcholine (ACh) receptors was investigated with HRP-α-BTX conjugates, after the cells membranes were permeabilized with 3-mercaptoethanol buffered rinses followed by a 3% saponin wash and at 21% deg. Muscle fiber staining potentials (HRP-α-Btx) were recognized intracellularly, electrodes placed close to endplates located using Hoffman modulation contrast optics. At all ages examined HRP frequency was lower at dystrophic than at normal endplates. In dystrophic muscle fiber membranes time constants (HRP-α-Btx) were recognized intracellularly. E-PTA also stains a prominent band beneath the submembranous density and separated from it by a space. The specialized membrane area is closely associated with small clear synaptic sites of myogenic cells. At sites of early nerve-muscle contact, patches are more numerous and larger. Often the external coating extends a greater distance into the extracellular space forming bridges with a sparse coating on the surface of growing nerve fibers. In more highly developed junctions, the external layer and submembranous density occupy the entire postsynaptic region. This study reveals the presence of external and internal specializations at regions of high ACh density and at sites of initial nerve-muscle contact. These specializations may be involved in the localization of positions of AChR in the membrane or contain components which establish these areas as preferential sites of nerve contact. (Supported by the Osserman Fellowship of the Myasthenia Gravis Foundation and NIGER BRS 78-13729.)


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Supported by the Medical Foundation and United States Public Health Service grant NS11160-06.
1615 THE EFFECT OF TEMPERATURE ON MEPP AMPLITUDE DISTRIBUTIONS AT THE MOUSE DIAPHRAGM. C.G. Carlson*, C.G. Muniak*, and F. Liado* (SPHC: C. Edwards). - Dept. of Physiology, Upstate Medical Center, Syracuse, NY 13210

Kriebel, Liado, and Matteson (J. Physiol. 262, 1976) observed a class of small spontaneous potentials (miniature end-plate potentials, s-MEPPs) in muscle fibers of the mouse diaphragm. These s-MEPPs are generally 1/10 to 1/15 the amplitude of normal end-plate potentials (m-MEPPs) for muscle fibers, and are relatively resistant to the depressive effects of Botulinum toxin on spontaneous frequency. In these experiments we determined the effect of low temperature on the relative frequencies of s-MEPPs and m-MEPPs.

Between 11 and 30°C, the overall MEPP frequency (s-MEPPs and m-MEPPs combined) showed a positive temperature dependence (c.f., Liley, J. Physiol. 132, 1956). The MEPP frequency increased from about 0.5 sec⁻¹ at 15°C to 1.4 sec⁻¹ at 25°C. At temperatures above 20°C, the MEPP distributions were similar to those observed at higher temperatures (23-27°C). MEPP amplitudes 1 to 4 times the s-MEPP showed distributions that skewed towards the smaller MEPP amplitudes (the "skew class"). MEPP amplitudes 5 to 20 times the s-MEPP showed distributions that were bell-shaped (the "bell-shaped" or m-MEPP class). Below 16 to 17°C, the s-MEPPs and corresponding "skew class" of MEPPs virtually disappeared in relation to the number of MEPPs in the bell-shaped part of the distribution. This indicates that in the range of temperatures close to 11°C, the frequencies of s-MEPPs and "skew class" MEPPs are more sensitive to a reduction in temperature than the frequency of the larger MEPPs in the "bell-shaped" class. Although s-MEPPs at 11°C were smaller in time course than MEPPs at higher temperatures (23-27°C), there was no obvious effect of temperature on either the s-MEPP or m-MEPP amplitude.

In some cells an optical temperature for the release of s-MEPPs was seen between 25 and 30°C. This is consistent with the observation that the percentage of m-MEPPs was smaller than at 3°C. These results provide further evidence that s-MEPPs and m-MEPPs are released by relatively independent mechanisms. (Supported by NIH Grant #11-15240)

1616 THE EFFECT OF A SODIUM IONOPHORE, MONENSIN, ON NEUROMUSCULAR TRANSMISSION. Milton F. Charlton, Brian J. Farnellf and Harold L. Atwood. Zoology Dept., University of Toronto, Toronto, Canada, M5S 1A1

Mounting evidence indicates that intracellular Na+ may playa role in the modulation of the transmission of acetylcholine (ACh) in the synapse. Increases in intracellular Na+ caused by Na+ pump inhibitors (Proc.Roy.Soc.B, 170,381-399), prolonged stimulation (Br.Res., 113B, 198-204), direct injection (Br. Res., 134, 367-371) or loading by liposomes (P.N.A.S.U.S.A., 75, 5214-5216) can result in increases in both spontaneous and evoked release. In this study Na+ loading was attempted by the use of a carboxyl Na+ ionophore, Monensin (Mon), which shows moderate selectivity against K+ and very little complexation with Ca++. (Ann.Rev. Biochem., 46, 501-530). Mon (3-7uM) caused an increase in excitatory post synaptic potentials (EPSP) of up to 250% within 15 min of application to crayfish opener, stretcher and abdominal extensor muscles (12-15°C). EPSP's were not increased by Mon in saline containing only 1/3 [Na+]. It is therefore likely that Mon is effective by increasing intracellular Na+. The effects of the ionophore Mon in the phasible following washout but return to normal transmission took longer than the initial facilitation.

The effect of Mon on spontaneous release of transmitter was tested in the frog cutaneous pectoralis muscle by recording miniature endplate potentials (mMEPPs). The frequency of mMEPPs was increased by over 2000% during an 8 min application of Mon (at 21°C). mMEPP frequency was still increased 1000% 13 min after washout of Mon. The membrane potential of muscle cells in either animal was not affected by Mon.

Neurotransmission for prolonged loading, the application of Mon is simple, rapid, reversible, does not involve poisons, does not require stimulation and can be used on cells of all synapses. The observation that the intracellular Na+ can modulate transmitter release and indicate that Mon may be a valuable tool in the study of the mechanism of this phenomenon.

1617 SHIFTS IN THE MULTIPLE FORMS OF ACETYLCHOLINESTERASE IN CHICK EMBRYONIC SKELETAL MUSCLE IN VIVO AND IN VITRO. C. Michael Cisson, Carole R. McQuarrie#, Mark G. McNamara, and Barry W. Wilson. University of California, Davis, CA 95616

Experiments in our laboratory to understand the progression of development of multiple forms of acetylcholinesterase (ACHE). The multiple forms in 11- and 18-day-old chick embryonic pectoral muscle and cultured muscle grown aneurally for 30 days were determined by centrifugation on a linear 5-20% sucrose gradient. These results reinforce the idea that there is a shift from small to larger molecular forms of ACHE during development and the relative resistant to the depressive effects of Botulinum toxin on spontaneous frequency. In these experiments we determined the effect of low temperature on the relative frequencies of s-MEPPs and m-MEPPs.

Between 11 and 30°C, the overall MEPP frequency (s-MEPPs and m-MEPPs combined) showed a positive temperature dependence (c.f., Liley, J. Physiol. 132, 1956). The MEPP frequency increased from about 0.5 sec⁻¹ at 15°C to 1.4 sec⁻¹ at 25°C. At temperatures above 20°C, the MEPP distributions were similar to those observed at higher temperatures (23-27°C). MEPP amplitudes 1 to 4 times the s-MEPP showed distributions that skewed towards the smaller MEPP amplitudes (the "skew class"). MEPP amplitudes 5 to 20 times the s-MEPP showed distributions that were bell-shaped (the "bell-shaped" or m-MEPP class). Below 16 to 17°C, the s-MEPPs and corresponding "skew class" of MEPPs virtually disappeared in relation to the number of MEPPs in the bell-shaped part of the distribution. This indicates that in the range of temperatures close to 11°C, the frequencies of s-MEPPs and "skew class" MEPPs are more sensitive to a reduction in temperature than the frequency of the larger MEPPs in the "bell-shaped" class. Although s-MEPPs at 11°C were smaller in time course than MEPPs at higher temperatures (23-27°C), there was no obvious effect of temperature on either the s-MEPP or m-MEPP amplitude.

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In cultures of myotomal muscle and spinal cord (SC) derived from Xenopus embryos SC neurites can trigger the development of a high density of ACh receptors along the paths of nerve-muscle contact (1,2) and electron microscopy reveals the presence of thin synaptic specializations normally found at autonomic nerve-muscle contacts (3,4). We have now examined whether neurites of dorsal root ganglia (DRG) and sympathetic ganglia (SG) interact with the cultured muscle cells in a similar way. These SC explants did not adversely alter the pattern of differentiation of muscle cells.

Muscle cells were plated at the same time as the nerve or shortly after the onset of neuritic outgrowth. After 2-4 days, when nerve-muscle contacts were apparent, ACh receptors were stained with fluorescent o-bungarotoxin in order to be able to visualize sites of high receptor density. Over 70% of SC-contacted muscle cells displayed some staining along the paths of contact whereas the corresponding values of DRG- and SG-contacted muscle cells were 9% and 5% respectively. Similar differences between SC- and SG-contacted muscle cells were also seen when both types of nerve were present together in the same culture chamber, thereby indicating that the SC explants did not adversely alter the culture medium. The few examples of staining associated with DRG- and SG-muscle contacts resembled the characteristic patches of stain seen on non-contacted muscle cells rather than the unique long bands of stain seen along some of the SC-muscle contacts. It is concluded that the accumulation of ACh receptors at SC-muscle contacts is triggered by a specific neural property which is lacking in the DRG and SG neurites.

Cultures containing muscle and DRG or SG explants were also examined by electron microscopy. Although the neurites approached the muscle fibers, they were rarely observed at these nerve-muscle contacts. The much more frequent occurrence of these synaptic specializations at sites of SC-muscle contacts (4) indicates that the SC neurites possess a specific property which is important for the establishment of synaptic contacts with muscle. This property is presumably the same as that which triggers ACh receptor accumulation at muscle contacts.

SYNAPSE FORMATION AT OLD AND DE NOVO ENDPLATE SITES. Ronald Ding*  

Re-inervation of old endplate sites and formation of de novo junctions at ectopic sites were studied in the cutaneous pectoris of the Rana pipiens using electrophysiology and light microscopy. Each muscle fiber studied electrophysiologically was marked intracellularly with Chicago Blue 68 so that it could be identified later after sectioning the extracellular spaces with nitroblue tetrazolium and for cholinesterase by the method of Karnovsky (J. Cell Biol. 23:217-232, 1964). Seven days or more after nerve crush, nerve terminal could be seen regrowing into and quickly filling old synaptic gutters. The nerve terminal had its fastest growth phase at postoperative times from 10 to 15 days. Up to 30 days after nerve crush, miniplate endplate potential (mepp) frequency per unit length of nerve terminal was appreciably lower than normal. Preliminary evidence showed an initial increase in quantal content per unit length of terminal, followed by a decrease and then another increase. Data obtained at postoperative delays of 30 days or more in general showed that the staining pattern of nerve terminal, mepp frequency and quantal content per unit length of terminal were all roughly equivalent to normal.

In other animals when the nerve was transposed to an endplate-free region of muscle and the endplate-containing portion discarded, de novo synapse formation occurred in ectopic regions. Compared to normal old re-inervated junctions these de novo junctions were more branched, were not as consistently aligned with the long axis of the muscle fibers, and were associated with more but smaller cholinesterase spots on muscle fiber. This distinct morphology persists for as long as 2 years. Mepps, endplate potentials, and contractions were all first seen at 4 weeks postoperative. Physiological similarities and differences between these synapses and those observed at re-inervated endplates will be discussed.

Supported by USPHS grant NS06232 to A. D. Grinnell, and an MBHE Fellowship to R. Ding.


The effects of acetylcholine and edrophonium on motor nerve endings were studied in the in vivo cat sciatic nerve synapse preparation. In the absence of edrophonium, 1.0 µg/kg i.a.* resulted in the appearance of stimulus-bound repetitive activity (SBR) and drug-induced activity recorded from the nerve. These neural responses were transmitted to the muscle; the former resulted in enhanced force of muscle contraction, the latter resulted in muscle fasciculations. The SBR persisted for 96 seconds and resulted in potentiation of contractile strength to 260% of control. Acetylcholine administered at 100 µg/kg i.a. also resulted in SBR and drug-induced activity though accompanied by a brief decrease in the force of muscle contraction. The SBR following acetylcholine persisted for only 15 seconds. Pretreatment with tetrodotoxin, 30 ng/kg i.a., of 30 to 60 seconds duration, resulted in complete elimination of SBR and drug-induced activity following acetylcholine but only partial decreases is SBR, drug-induced activity, and the enhanced contractile strength following edrophonium. Similar pretreatment with the calcium antagonists verapamil (10-100 µg/kg), D-600 (10-100 µg/kg) and LaCl3 (0.1-10 µg/kg) resulted in dose-related decreases in neural and muscle responses to edrophonium but had no effect on responses to acetylcholine. In order to study the interaction of these two compounds with AChE, we performed following administration of theophylline, a phosphodiesterase inhibitor. Theophylline (10-100 µg/kg) produced a dose-related increase in the neural and muscle response to edrophonium while it had no effect on responses to acetylcholine. It is concluded that the observed acetylcholine effects probably result from a sodium depolarization of the motor nerve terminal while the edrophonium responses probably result from both sodium depolarization and a cyclic AMP-mediated influx of calcium into the nerve ending.

(Supported in part by USPHS NS 12566)


Differences have been detected between junctional and extra-junctional acetylcholine receptor (AChR) from rats using serum from myasthenic patients (Weinberg and Hall (1979) PNAS 76:504). Titers against extrajunctional AChR were an average of 1.5 times higher than titers against junctional AChR. We have investigated this problem using three separate assays: inhibition of 125I-a-acetylcholine binding, conventional immunoprecipitation and a modification of immunoprecipitation developed in this laboratory (for details see: Dwyer et al., Clin. Exp. Immun., in press). Characterization with sera from 10 patients has been conducted. Our data indicate no systematic differences between the two types of receptor when titers are compared. Several patients show slightly higher titers against junctional AChR; the reverse is also found.

Inhibition assays detect differences between receptor. Unlike previous reports (Almon and Appel (1975) Biochem. Biophys. Acta 393:66), we can measure inhibition with junctional receptor in 50% of patients tested compared to 90% able to inhibit against denervated AChR. Maximum inhibition of toxin binding has been measured as high as 75-90% with some sera. When saturation curves are plotted with percent inhibition vs. amount of serum, both the slope of the line and the maximal inhibition is in most cases greater against nonjunctional receptor. This suggests that there is antibody in most myasthenic sera that recognizes a determinant on extrajunctional AChR close to the toxin binding site.

We have also observed that a certain fraction, approximately 10-15%, of either junctional or extrajunctional AChR is not precipitated by myasthenic IgG even at saturating antibody concentrations. This phenomenon occurs even when the modified procedure is used, which corrects for antibody precluded from binding AChR by the toxin label. This appears to be a consequence of microheterogeneity of AChR itself. Whether this heterogeneity exists in intact membranes or whether it occurs as a consequence of tissue disruption is not known.


The presence of multiple molecular forms of acetylcholinesterase (AChE) was examined in muscle cell cultures derived from pectoral muscle of 9 day quail (Coturnix coturnix japonica) embryos. Muscle cells were scraped from tissue culture plates, homogenized in 0.1M NaCl and 0.5% Triton X-100. Assay of gradient fractions for AChE activity and analyzed by velocity sedimentation on a linear sucrose gradient containing 0.02 M Korate buffer (pH 8.8) containing 1M NaCl and 0.5% Triton X-100. The homogenate was centrifuged for 30 minutes at 45,000 rpm in an SW 50.1 rotor and the supernatant collected and analyzed by velocity sedimentation on a linear sucrose gradient containing 0.02 M Korate buffer (pH 8.8). 1M NaCl and 0.5% Triton X-100. Assay of gradient fractions for AChE activity revealed three major peaks with sedimentation values of 21, 13 and 6.5 S. The 21S form made up to 15% of total AChE activity on the gradient. When fractions containing peaks of activity were re-run, they sedimented to their original position and gave rise to no new enzyme forms. Both culture medium and chick muscle cell cultures did not possess a large form of AChE or peaks of activity coincident with the 21S form. AChE isolated from quail muscle in vivo was found to possess three forms which correspond to the forms present in cultured quail muscle cells. Results were performed to compare the in vivo and in vitro 21S forms. When the two enzymes were sedimented in gradients containing reduced NaCl concentrations (0, 0.2, and 0.4M), both showed a tendency towards aggregation as reflected in broader peaks and higher S values. In addition both 21S forms were collagenase sensitive and acquired an increased sedimentation value of 22S when combined with the enzyme. These observations indicate that the in vivo and in vitro 21S AChE are similar and suggest the presence of a collagen-like tail on the enzyme.

Studies are underway to ascertain the relationship of the 21S form to the neuromuscular synapse and synaptosomes both in vivo and in vitro.

Aminoglycoside antibiotics are known to block neuromuscular transmission, but the mechanism of action is not clearly understood. Both pre- and postsynaptic blocking mechanisms have been implicated, but their relative contributions to the neuromuscular block has not been quantitatively determined. The effects of streptogramin on end-plate currents (EPC) were studied using the two microelectrode voltage clamp technique. Frog cutaneous pectoris muscles were used in all experiments. The EPC was decreased in amplitude by streptogramin at a concentration of 3 x 10^-6 to 3 x 10^-7M. The linearity of the current-voltage relationship was not appreciably altered by streptogramin, although slight curvature might occur especially at large negative potentials. The EPC was slightly prolonged in time course; for example at -70 mV, the time constant of decay was increased from 1.31 to 1.54 msec by 3 x 10^-5 streptogramin. No frequency dependent block was observed. The EPC's value for the block of EPC's under conditions of high quantum content was estimated to be 8.5 x 10^-5. Under conditions of low quantum content in the presence of high magnesium and low calcium, 3 x 10^-5 streptogramin decreased the quantum content by a different venom component than are the effects in vertebrate nervous systems, and the resulting physiological and morphological effects were examined. Application of BWSV causes a biphasic effect on excitable endplate potentials (EPSPs) evoked at a lobester neuromuscular junction where glutamate is the putative excitatory neurotransmitter. Tryptamine also reduced GABA mediated inhibitory post synaptic potential amplitudes (Friedman, et al.).

In the present study, I used conventional electrophysiological techniques to examine the effects of tryptamine on cholinergic transmission in the frog sartorius nerve-muscle preparation bathed in a modified (0.4mM Ca++) Ringer's solution. Tryptamine (0.1-0.5mM) caused an increase in EPP amplitudes. Since the average miniature EPSP amplitude was virtually unchanged, it seems that the action of tryptamine is a presynaptic effect which increases the mean number of quanta released by the nerve impulse. Higher tryptamine concentrations (≥10μM) depressed average EPSP amplitudes. Both effects were reversible upon washing. These results indicate that the effects of tryptamine at a cholinergic synapse are similar to those observed at presumably glutamatergic synapses of the lobester neuromuscular junction.

P. Friedman, R.N., Shank, R.P., and Freeman, A.R. Brain Res. (submitted).

This work was supported by a Lady Davis Foundation Fellowship and the U.S.-Israel Binational Science Foundation.


Lobster stretcher muscles were treated with black widow spider venom (BWSV) and the resulting physiological and morphological effects were examined. Application of BWSV causes potentiation of EPSP's and IPSP's and a massive increase in the miniature EPSP frequency was still high) contained vesicles, but bathing solution.

potentials are abolished, miniature EPSP frequency decreases to zero), most terminals were depleted of vesicles, and large infoldings of the nerve terminal leading to vesicle dispersion, vesicle-fusion, vesicle-expulsion, and vesicle-tubulosis.

"Giant minis" may be caused by the release of clumped or prepulsed vesicles. The morphological results are similar to those seen following BWSV treatment of vertebrate neuromuscular junctions, even though the effects in crustaceans are mediated by a different venom component than are the effects in vertebrates [Fritz et al. Soc. for Neurosci. Abs. (1978) # 369].

β-Bungarotoxin Stimulates Acetylcholine Synthesis in Rat Diaphragm. Cameron R. Anderson, Michael W. Newton*, Donald J. Jenden, Dept. Pharmacology, UCLA School of Medicine, Los Angeles CA 90024

β-Bungarotoxin (β-Btx) is a presynaptically acting polypeptide neurotransmitter with Ca++-dependent phospholipase A activity. We have observed that β-Btx (140 ng/ml) caused a large increase in tissue levels of acetylcholine (ACH) in rat hemidiaphragms. Relative to control values (3.5 pmol/mg wet wt), a 100% increase in tissue ACH was measured within 45 min of toxin exposure of an unstimulated muscle in the absence of a cholinesterase inhibitor. Subsequent studies indicate that uptake of extracellular choline (Ch) and Ca++ are both necessary to produce the rise in tissue ACh content. Fan-shaped segments of hemidiaphragm (wet wt: 50-90 mg) of 90-150 g male rats were treated for 30 min with β-Btx (140 ng/ml) in an everted (15 mm) Krebs-bicarbonate medium at 37°C. After three 10 min periods of indirect (10 Hz) stimulation and a final 15 min rest period the released ACh and the tissue ACh were assayed by gas chromatography mass spectrometry. When [2H4]-ACh (1 am) was added to the Krebs medium, very little label was incorporated into ACh in either toxin treated or control diaphragms. This may be due to the dilution of labelled precursor by endogenous Ch, which is released by diaphragm at rates in excess of 1.0 pmol min^-1-mg wet wt^-1. The average Ch concentration of diaphragm is 50 pmol/mg wet wt. Using [2H4]-Ch (10 am), 55 pmol of [2H4]-ACH (0.74 pmol per mg wet wt) was retained by control preparations, while 249 pmol of [2H4]-ACH (4.1 pmol per mg wet wt) was present in toxin treated samples. Total ACh levels (pmol/mg wet wt) were 9.4 in toxin treated vs 2.3 in controls. Hemicholinium-3 (10μM) blocked the increase in tissue levels of ACh caused by β-Btx and reduced stores to 0.7 pmol per mg wet wt. When Ca++-free Krebs supplemented with SrCl2 (2 mM) or EGTA (1mM) were used, β-Btx did not cause an increase in tissue ACh levels. Evoked ACh release in both the SrCl2 and EGTA containing media was inhibited. We conclude that external sources of Ch and Ca++, of which the latter may be necessary for the toxin phospholipase activity, are required for the increased ACh levels following β-Btx treatment of the rat diaphragm. (Supported by USPHS grants NS-05753 and MH-17691)

TRYPAMINE-INDUCED ALTERATIONS OF ACETYLCHOLINE RELEASE AT A NEUROMUSCULAR JUNCTION. Richard N. Friedman* (SfN: R. Rahemt (submitted). The EPC was slightly prolonged in time course; for example at -70 mV, the time constant of decay was increased from 1.31 to 1.54 msec by 3 x 10^-5 streptogramin. No frequency dependent block was observed. The EPC's value for the block of EPC's under conditions of high quantum content was estimated to be 8.5 x 10^-5. Under conditions of low quantum content in the presence of high magnesium and low calcium, 3 x 10^-5 streptogramin decreased the quantum content by a different venom component than are the effects in vertebrate nervous systems, and the resulting physiological and morphological effects were examined. Application of BWSV causes a biphasic effect on excitable endplate potentials (EPSPs) evoked at a lobester neuromuscular junction where glutamate is the putative excitatory neurotransmitter. Tryptamine also reduced GABA mediated inhibitory post synaptic potential amplitudes (Friedman, et al.).

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Recent histological studies have shown that chronic denervation causes an increase in the size of the end-plate region. This increase is usually accompanied by an increase in the number of nerve terminals. The experimental muscles showed an approximately 5-fold increase in the number of nerve terminals following the elimination of activity (disuse), we made comparisons of synaptic function between disused and normal (contralateral) soleus muscles in rats.

Muscle disuse was induced by tetrodotoxin (TTX) impregnated silicone cuffs around the sciatic nerve. Conduction block was confirmed by the absence of spontaneous end-plate potentials (m.e.p.p.) in the resting nerve. The end-plate potentials were observed in the disused muscle in the absence of spontaneous activity after 8-14 days of disuse.

In the seven animals studied, the mean m value from the disused side was significantly greater than that from the control side (P<0.01). The average difference in m.e.p.p. frequency between the two sides was noted. In five additional animals, the effect of a control cuff (no TTX) on the disused side (P<0.05), the average difference being 80%.

The left sartorius muscle of anesthetized adult Rana pipiens was exposed, the sartorius nerve crushed, and a longitudinal cut made through the nerve to expose the fibers in the lateral half of the muscle. When the motor axons regenerated, they would encounter only the intact medial half of the muscle, which was treated with 0.025% trypsin and grown under tissue culture conditions. The myoblasts fused to form multinucleate myotubes, which were treated with 10-8 M vinblastine to form myoballs. The myoballs were sucked onto the tip of a glass pipette, the membrane was ruptured, and the cell placed in a recording chamber and superfused with an oxygenated, low calcium and high magnesium buffered saline solution.

The m.e.p.p. frequency was measured in the resting nerve and in the disused nerve after 8-14 days of disuse. Approximately 50 seconds of spontaneous m.e.p.p.s were obtained after 8-14 days of disuse. In the seven animals studied, the mean m value from the disused side was significantly greater than that from the control side (P<0.01). The average difference in m.e.p.p. frequency between the two sides was noted.

We conclude that chronic disuse leads to an increased evoked release of transmitter as well as an increased spontaneous release of neurotransmitter. This is consistent with the hypothesis that under such circumstances, the functional end-plate area becomes enlarged. (Supported by USPHS grants NS 11132 and NS 10319)

Single-channel current fluctuations were observed for acetylcholine (ACH)-sensitive channels in cultured embryonic rat myotubes. The current fluctuations were observed in a cell cluster where a carbon fiber electrode containing 1 or 10 mM carbamyl choline was pressed against the cell membrane. Rectangular current-fluctuations caused by the agonist-activated ACh channels were observed with a pipette-to-membrane seal resistance of 20 MO or greater. For 10-day old cultures, cells held at a membrane potential of ~90 mV, gave channel junctions of 4.4 pS. Assuming a reversal potential of 0 mV, this leads to a value for the unit-channel conductance of 46 pS. Open-channel durations were exponentially distributed, with a mean open-time of 3.3 msec. As a convenient means of analyzing channel properties, the fluctuation records were processed by a Fourier transform program. The results were determined by Lorentzian, and the open-channel durations could be extracted even for records so dense that individual-channel events are unresolved. The surfaces of the myotubes were probed for variations in acetylcholine sensitivity. The regional intensity channel activity was correlated with regions of high receptor density as measured by fluorescence microscopy with rhodamine labeled α-bungarotoxin. The direct recording of ACh-sensitive single-channels together with the analysis of membrane noise, permits the quantitative mapping of the spatial distribution of ACh receptors and the characterization of the parameters of the ACh sensitive channels.


The innervation of skeletal muscle at the neuromuscular junction is known to be cholinergic. Since choline acetyltransferase (ChAc) catalyzes the synthesis of acetylcholine, high ChAc activity has been detected in the ventral horn, the ventral root and the nerves that innervate skeletal muscle. Until recently we have visualized ChAc in motor neurons of bovine spinal cord with an immunofluorescent method using guinea pig anti-ChAc serum and we have localized ChAc in the motor neurons of rabbit spinal cord (Brain Res. 146: 221, 1978) by the sequential incubation of the sections with rabbit diaphragm were stained histochemically for cholinesterase (ChE) using 5-bromoindoxyl acetate as the substrate. The immunoperoxidase staining involved the sequential incubation of the sections with pig IgG and guinea pig peroxidase-antiperoxidase (PAP). The innervation of skeletal muscle at the neuromuscular junction is known to be cholinergic. Since choline acetyltransferase (ChAc) catalyzes the synthesis of acetylcholine, high ChAc activity has been detected in

DEPRESSANT EFFECTS OF MORPHINE AND MEPERIDINE ON NEUROMUSCULAR TRANSMISSION IN RAT AND HUMAN MYASTHENIC MUSCLES. Yong I. Kim, James F. Howard and Donald B. Sanders. Dep't of Neurology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

The depressant actions of narcotic analgesics, morphine (MO) and meperidine (ME), on neuromuscular transmission were investigated in vitro in forelimb flexor digitorum longus muscles of normal rats and patients with myasthenia gravis (EAMG) and in intercostal muscle biopsies from patients with myasthenia gravis (MG).

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ME and MO produced a similar reduction in MEPP and EPP amplitudes. At maximum therapeutic concentrations (MO: 0.03-3 μM, ME: 0.7 μM), both drugs showed a dose-dependent effect on neuromuscular transmission. At 10-40 times the therapeutic concentration only ME produced an apparent neuromuscular depression. At very high concentrations (160 μM), both drugs decreased the amplitude and maximum rates of rise and fall of MEPP, suggesting an effect on the ionic conductances of muscle membranes. Isometric twitch tension was significantly reduced by both drugs in muscles in which neuromuscular transmission was partially blocked by d-tubocurarine. The concentration of MO required to produce a given reduction of twitch tension was greater than that of ME. Similar depressant effects of MO and ME on neuromuscular transmission were found in muscles from rats with EAMG.

In intercostal muscles from patients with MG, both MO and ME depressed MEPP and EPP amplitudes in a dose-dependent manner. Reductions in MEPP and EPP amplitudes were observed with 30 μM MO and 70 μM ME but the quantum content of evoked transmitter release was not altered, indicating the absence of a direct action of MO and ME on host motor axons. At therapeutic concentrations, both drugs had no inhibitory effect. At 100 times the therapeutic concentration, MO (30 μM) still had no inhibitory effect, but ME (70 μM) significantly impaired the neuromuscular transmission. These results demonstrate that meperidine has a greater depressant effect on neuromuscular transmission in vitro than does morphine, but that these effects are seen only at concentrations significantly greater than are achieved clinically.
ACh RECEPTOR CHANNELS BEGIN TO OPEN WITHIN 10 μsec AFTER AGONIST IS APPLIED. Mauri E. Kroogse, Henry A. Lester, Menachem N. Nuss, Jeanne M. Herbon, Robert B. Measeyena and Bernard F. Gainera (SPOR: W. P. Agnew). Division of Biology, California Institute of Technology, Pasadena, CA 91125 and College of Phys- 

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Nerve terminals were no longer seen and the end-plate did not contract when the nerve stump was stimulated. At this stage, Schwann cells still occupied the synaptic clefts, but now ridges appeared on the cytoplasmic face of the Schwann cell membrane. Like the active zones of nerve terminals, these ridges lay directly over the functional clefts. However, no rows of particles marked these sites on the Schwann cell membrane. Examination of this section from paired muscles confirmed that Schwann cell processes formed these ridges and that these ridges are over the functional folds. However, no other specialized cytoplasmic structures or clustering of organelles in these regions of Schwann cells were found. Mapping of the Schwann cell membrane to the contours of the functional folds suggests attachment of the Schwann cell to the muscle or its basement membrane in the location to which nerve terminals are also thought to be attached.

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1640 A COVALENTLY BOUND PHOTOSENSORIZABLE AGONIST AT ELECTROPHORUS ELECTROPLAQUES: EQUILIBRIUM, KINETICS, AND STOICHIOMETRY. H. A. Lester, M. E. Efron and M. M. Marx. Division of Biology, California Institute of Technology, Pasadena, CA 91125; N. H. Wassermann and B. F. Ehrlicher, Department of Microbiology, Columbia University, New York, NY 10032.

After disulfide bonds are reduced with dithiothreitol, trans-3-(α-bromomethyl)-3′-[(α-trimethylammonium)methyl]azobenzene (tethered-QBr) alkylates the QBr binding site of the receptor. The membrane conductance induced by this "tethered agonist" shares many properties with that induced by reversible agonists. Equilibrium conductance increases with agonist concentration; membrane conductance is not reversed; the voltage sensitivity reassembles that seen with 50 μM carbachol. Voltage-jump relaxations follow an exponential time course; the rate constants are about twice as large as those seen with 50 μM carbachol and have the same voltage and temperature sensitivity. With reversible agonists, the rate of channel opening increases with the frequency of agonist-receptor encounters; but with tethered-QBr, this rate depends only on intramolecular events. In comparison to the conductance induced by reversible agonists, QBr-induced conductance is at least tenfold less sensitive to competitive blockers, such as tubocurarine, and roughly as sensitive to "open-channel blockade" by QX-222.

Light-Flash experiments with tethered-QBr resemble those with the reversible photosensitizer agonist, Bis-Q: the conductance is increased by light + trans photosensitization and decreased by trans + cis photosensitization. As with Bis-Q, light-Flash relaxations have the same rate constant as voltage-jump relaxations. Receptors with tethered-QBr have a channel duration severalfold briefer than with the tethered trans isomer. By comparing the agonists, the cis/trans ratio, we conclude that each channel's activation is determined by the configuration of a single tethered QBr molecule. The QBr-induced conductance (at high time constants, several hundred msec) which can be partially reversed by flashes. The similarities suggest that the same rate-limiting step governs the opening and closing of channels for the tethered and tethered agonists. Therefore, this step is probably not the initial encounter between agonist and receptor molecules.

Supported by the Muscular Dystrophy Association, the NIH (RO1NS-272 and Grant NS-11756) and the NSF (Grant PCM 74-2140).
125I-α-BTX site density†

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<th>age (Animals)</th>
<th>Genotype</th>
<th></th>
<th>Morphology</th>
<th>L251-α-BTX site density†</th>
<th>per μm²</th>
<th>per μm²</th>
<th>per μm²</th>
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<td></td>
<td>normal</td>
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<td>total thickened pjm/1st cleft</td>
<td>± 0.66±0.03</td>
<td>± 1.33±0.12</td>
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<tr>
<td>1d(3)</td>
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<td>± 1.00±0.04</td>
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<tr>
<td>5d(3)</td>
<td>± 1.23±0.07</td>
<td>± 0.66±0.03</td>
<td>± 0.67±0.10</td>
<td>± 0.82±0.11</td>
<td>± 1.34±0.11</td>
<td>± 1.00±0.04</td>
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<td>± 1.27±0.08</td>
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<tr>
<td>&gt;4mo(2) +/dy</td>
<td>± 1.32±0.65</td>
<td>± 1.33±0.12</td>
<td>± 1.76±0.11</td>
<td>± 1.27±0.08</td>
<td>± 1.76±0.11</td>
<td>± 1.00±0.04</td>
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</table>

*normalized to site density per thickened pjm in normal adult.
†± SD
Monoclonal Antibodies to the Nicotinic Acetylcholine Receptor from Rat Muscle. Jeffrey Boone Miller and Zach W. Hall. (SPON: M. Stryker). Department of Physiology, University of California, San Francisco, CA 94143

We have used the hybridoma technique to prepare monoclonal antibodies to acetylcholine (ACh) receptor purified from denervated rat skeletal muscle. Both immunized rats and mice were used to produce antibodies-producing cells. BALB/c mice were immunized by two injections of either 0.5 μg ACh receptor in complete Freund's adjuvant or 10-50 μg of receptor that was bound to cobrotoxin-Sepharose and treated with glacial acetic acid. Rats were immunized with four 1 μg injections of receptor over two months. In all cases, animals produced detectable serum antibodies to the ACh receptor. Spleen cells from immunized animals were fused with cells from a mouse myeloma line, NS-1, and cultured in a medium that selected for hybrids. Anti-ACh receptor antibodies that were secreted into the culture medium by the hybrid cells were detected with a solid phase radiolmmunoassay that required only 1 ng of muscle receptor as a target antigen.

Anti-receptor producing hybridomas were detected at a low frequency (less than 1 in 1000) among the six hybridomas lines that secrete anti-receptor were isolated and have been continuously cultured. Of these, three are derived from mouse spleen/NS-1 hybrids and secrete IgM, while three are rat/mouse hybrids that secrete IgG. Antibodies from all six lines did not distinguish junctional and extra-junctional forms of the rat muscle receptor, and did not cross-react with the purified receptor from Torpedo 

This work was supported by grants from the Muscular Dystrophy Association and the N.I.H., and by a postdoctoral fellowship from the Muscular Dystrophy Association to J.W.H.
PROPERTIES OF SINGLE CHANNELS IN TISSUE CULTURED SKELETAL MUSCLE. C. Gary Reddick* and Zach W. Hall, Dept. of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214.

Using the technique of Neher et al. (Pflug. Arch. 375: 219, 1978) we have recorded the currents produced by single nicotinic channels in response to nerve stimulation in cell populations. In myotubes, the single channel conductance, when activated by suberyldicholine in Hanks' solution, had a positive temperature coefficient of about 10 kcal/mole. We saw no evidence of a levelling off of conductance with increasing temperature as reported by Fleischbach and Lass (J. Physiol. 280: 527, 1978).

The conductance values are consistent with the values obtained by noise analysis on normal and vinblastine treated cultures (Sachs and Lecar, Biophys. J. 17: 129, 1977; Nature 266: 214, 1977).

Histograms of the open channel durations were exponential implying a Poisson process of channel closing. The time constants with suberyldicholine activation were about 25 msec at 17°C and 6 msec at 36°C. The activation enthalpy, based on Arrhenius plots was about 10 kcal/mole. Confirming the results of Neher et al. (ibid), mobility of AChR found in aggregates may be associated with the anchorage of AChR to the myotube cytoskeleton. Medium conditioned by the neuronal cytoskeleton underlying the spatial organization of AChR with the anchorage of AChR to the myotube cytoskeleton.

Myotubes were rapidly extracted by detergent (T 1/2 <1 min.) Further differentiation of cultured myotubes is marked by aggregation with the anchorage of AChR to the myotube cytoskeleton. Rat myotubes at about 10 d. Whether receptors with the immunological properties of EJR (t½=32 h) but by ED 20 turnover in the clusters of the two components of facilitation, after correcting for depression, were not affected over this 24-fold range of Ca++ concentrations.

We propose that anchorage of AChR to the muscle cytoskeleton is nearly the same as for receptors in adult skeletal muscle. The selective effects of Sr**, although present, appeared to decrease with increasing quantal content, while the magnitude of augmentation was still increased by Ba++.

The differential effects of Sr** and Ba++ suggest that some of the underlying factors affecting these processes are different. Supported by NIH grants NS06581 and NS10277.
end-plates, presumably secondary to reduction in the number of blocked preparations was not significantly different from functional ACh receptors, and support the concept that EAMG is control values.

The mean number of ACh quanta released per nerve impulse in Mg²⁺-reduced twitch tension of curarized muscles; (6) The carbamyl-tension reduction; application of 4-aminopyridine restored the amplitude was reduced to 25 to 50% of control values; (2) The receptor protein (AChRP) and a booster injection of 10 to 15 μg autoimmune myasthenia gravis (EAMG), produced by immunization with Y. I. Kim* (SPON: R.N. Johnson). Dept, of Neurology, Univ. of Nebraska.

IN THE ANIMAL MODEL OF MYASTHENIA GRAVIS. Donald B. Sanders and Mary B. Rheuben and Ann E. Rammer. Dept. of Biology, The Flow of Cytoplasm around the nerve extensively in the adult. There is a difference in the complexity of structure from one end of a junction to another, with the greatest development of postsynaptic and glial processes at the end first contacted by the nerve.

Junctions formed by a particular motor neurone are relatively uniform in length and complexity. Differences are seen between the populations of muscle fibers innervated by different neurones. On the basis of anatomical and physiological evidence, the developing muscle fibers are electrically coupled. Further development consists of increase in size and quantity of the pre- and postsynaptic structures and glial specializations.

Supported by NIH 5R01 NS13700 and NSF BNS 75-18569.

NEUROMUSCULAR JUNCTION

During development of the flight muscles of the moth Manduca sexta, synapses are formed de novo on maturing fibers. In the last week of pupation, increasingly large junctional potentials can be recorded. Intracellular recordings were made from fibers of the dorsal longitudinal muscles, each bundle of which is innervated by a single separate motor neuron, and measurements made of endplate potential amplitude and time course, and response to multiple stimulation. Samples were then removed and prepared for thin section, freeze-fracture, and scanning microscopy. Early in the last week, we record small, long duration synaptic potentials that fatigue readily. junctions are characterized by having postsynaptic structures seen either as small patches of particles on the external leaflet in freeze-fracture or small areas of electron dense membrane opposite the nerve in thin section. The membrane of the nerve terminal is specialized into plaque-like areas similar to those in adult junctions but smaller; there are no clusters of large particles in the center of the plaques in the cytoplasmic leaflet. In thin section the plaque area is seen as a region where the nerve membrane parallels the postsynaptic membrane, and the cluster of particles is in the same location as the presynaptic dense body or active zone. There are a few scattered or clumped, large round particles that lie both within the postsynaptic patches and adjacent to them. They appear to correspond to desmosome-like structures in thin section. Like similar structures found in other developing synapses, they may function in anchorage of nerve terminal to muscle fibers during the initial stages of apposition. Glial cells have a large volume of cytoplasm above the nerve terminal; they begin to form complexes around the muscle fibers around the nerve extensively in the adult. There is a difference in the complexity of structure from one end of a junction to another, with the greatest development of postsynaptic and glial processes at the end first contacted by the nerve.

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Supported by NIH 5R01 NS13700 and NSF BNS 75-18569.

FURTHER ELECTROPHYSIOLOGICAL STUDIES OF NEUROMUSCULAR TRANSMISSION IN THE ANIMAL MODEL OF MYASTHENIA GRAVIS. Donald B. Sanders and Yong I. Kim* (SPON: R.N. Johnson). Dept. of Neurology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

Neuromuscular transmission defects were examined in forelimb flexor digitorum longus (FDL) muscles from rats with experimental autoimmune myasthenia gravis (EAMG), produced by immunization with an initial subcutaneous injection of partially purified acetylcholine receptor protein (AChRP) and a booster injection of 10 to 15 μg AChRP given two weeks or more before the experimentation. Results show: (1) AChRP concentration (MEPP) amplitude was reduced to 25 to 50% of control values; (2) The resting membrane potentials and MEPP frequency were unchanged; (3) End-plate potential (EPP) and MEPP amplitudes were reduced the same amount; (4) The waveform of indirectly elicited muscle action potentials (MAPs) was normal; (5) Curare sensitivity of EAMG muscles was markedly augmented as measured by twitch tension reduction; application of 4-aminopyridine restored the reduced twitch tension of carusized muscles; (6) The carbamyl-choline-induced depolarization of EAMG end-plates was found to be significantly larger than that seen at normal end-plates; (7) The mean number of ACh quanta released per nerve impulse in Mg²⁺ blocked preparations was not significantly different from control values.

These studies confirm that the major defect of neuromuscular transmission in EAMG results from postsynaptic abnormalities at the end-plates, particularly in the number of functional ACh receptors, and support the concept that EAMG is primarily a postsynaptic disorder. EAMG in rats serves as a reasonable model for the human syndrome, in which a similar defect of neuromuscular transmission is present.

(Supported by NIH Grant NS-12905 and a center grant from the Muscular Dystrophy Association)

AN ACETYLCHOLINE RECEPTOR AGGREGATION FACTOR IS RELEASED FROM SYMPATHETIC AND SPINAL CORD NEURONS. A.E. Schaffner,* R.L. Schwan,* Z. Vogel,* and M.P. Daniels. NHLBI, NIH, Bethesda, MD 20205

Neuromuscular synapse formation is characterized by a redistribution of acetylcholine receptors (AChR) which result in receptor localization at the postsynaptic region of the muscle plasma membrane. Recently it was reported that sympathetic ganglia x gloma hybrid cells, if cultured with cultured muscle cells release a factor which increases the number of AChR aggregates on cultured striated myotubes, independent of new receptor synthesis (1). We now have evidence for release of such a factor by cultured primary cell cultures. Cultures of rat superior cortical ganglion (SCG) cells for 4 days with rat fibroblasts resulted in a 2.6 fold increase in the number of AChR aggregates per myotube (A/M) over control cultures which contained myotubes alone. The A/M was determined by staining cultures with a monoclonal antibody labeled-α-bungarotoxin (TM Tr A-BT) and counting fluorescent patches and myotubes. Incubation of myotube cultures for 24 hrs with conditioned medium (CM) from SCG cultures grown in L-15 medium without bicarbonate and containing only neurons ("adrenergic condition") caused a 1.8-2.6 fold increase in A/M over cultures which received control medium. Day incubation with this medium yielded a 2.8 fold increase. CM from SCG cultures grown in bicarbonate buffered medium, allowing growth of glial cells ("cholinergic conditions"), caused a 3.4 fold increase in A/M. However, CM from SCG cells cultured without neurons did not increase the A/M in myotube cultures. ACHR on myotubes were stained with TRM A-BT before application of CM. The cholinergic" CM caused a 2.1 fold increase in the A/M, indicating that the factor caused incorporation into aggregates of ACHR already on the muscle cell surface. Receptor aggregation activity was not blocked when myotubes were exposed to 10 μg/ml cycloheximide; thus the factor was effective in the absence of protein synthesis. Six day chick embryo spinal cord neurons were maintained in a Metrizamide density gradient and 3 fractions maintained in culture. CM from a fraction with a high ratio of choline acetyltransferase to lactate dehydrogenase (LDH) caused a 2.4 fold increase in A/M while fractions with low CAT/LDH activity caused only a 1.3-1.5 fold increase.

Studies in progress may indicate whether release of an aggregation factor is limited to cell types which can form cholinergic synapses.


Studies on the activation of depolarization-secretion coupling by divergent cations have generally been based on the mathematical framework of Hemi-Michais-Menten (HMM) enzyme kinetics. Such an interpretation assumes that the responses mediated by the active cations are Ca(++)-dependent and that the affinity of cation binding is independent of the cation. More recently, studies using the pharmacological tools of channel blockers and inhibitors of cholinesterase have shown that the cation dependence of the response is complex and may involve multiple channels. The purpose of this study was to re-examine the traditional mass action against therapy concept using conventional electrophysiological methods. Lanthanum (La) in micromolar concentrations has been shown to antagonize irreversibly evoked transmitter release by blocking Ca(++) entry into nerve endings. Such an effect has been confirmed in this study. In addition, however, a small elevation in the extracellular Ca(++) concentration in the presence of La caused an immediate, forceful increase in evoked transmitter release. It can be demonstrated that this effect of Ca(++) is not due to displacement of La(3+) by Ca(++) or to the effect of Ca(++) on evoked transmitter release. These results suggest that the effect of Ca(++) on evoked transmitter release is not mediated by a direct interaction between the two cations. The simplest explanation appears to be that increasing the external Ca(++) concentration surmounts the La(3+) antagonism by 'mopping-up' small external receptor sites. This interpretation is consistent with the observation that the following should hold: a) the partial agonist, Sr(2+) (which must occupy a large fraction of the binding sites of the receptor sites) could be antagonized by La(3+) and an increase in the external Ca(++) concentration should not restore transmitter release; b) comparison of equal levels of transmitter release by Ca(++) and Sr(2+) should provide an affinity constant for Sr(2+) that is similar to the value determined for Sr(2+) as an antagonist of Ca(++)-mediated release. The observed behaviour is in accord with these predictions. These results suggest that certain activities of La(3+) exist at frog motor nerve terminals and demonstrate that the mathematical approach of modern drug receptor theory is more appropriate than HMM kinetics when analyzing the agonist effects of divalent cations on transmitter release.


(+)Anatoxin (ATX), a stable toxin in the presence of juncational acetylcholinesterase (AChE), is a biliary tachylic amine having potent anticholinergic activity as evidenced by decreased acetylcholine sensitivity. ATX was shown by biological assay on frog rectus abdominis muscle to be 10-fold more potent than carbacholamine. Frog sartorius endplates demonstrated pharmacological effects as revealed by initial electrophysiological studies. The amplitude of epc without, however, changing the epc's voltage and acetylcholine (ACh). Iontophoretically applied to the endplate region, ATX was shown by biological assay on frog rectus abdominis muscle to be 15 minutes. ATX caused no alteration of the time course of the endplate current (epe) and at high concentrations, i.e., 1μM, it depresed the peak amplitude of the epc without affecting the epe. The epe was reduced over time at these concentrations, and 90% reduction in epc peak amplitude was recorded, there was no change in the equilibrium potential, which is similar to that of the natural agonist acetylcholine (ACh). Iontophoretically applied to the endplate region, ATX induced an inward current whose similarity to that of ACh disclosed that the single channel conductance and lifetime were unaltered. The two agents, ACh and ATX, applied to the same endplate region using a double-barreled microperifused disclosed, however, a slower onset and offset for ATX as compared to ACh, this effect was mostly related to the inability of ACh to hydrolyze ATX. Because of its depolarizing action, the toxin markedly decreased the amplitude of the miniature eps while producing a marked presynaptic effect, increasing the rate of release by several fold during iontophoretic application of ATX. At similar concentrations, ATX inhibited 60% of the binding of [3H]ACh to the membrane bound ACh receptor isolated from the electric organ of the Torpedo californica. ATX reduced the rate as well as equilibrium binding of [3H]α-bungarotoxin. ATX also blocked the binding of [3H]-niuacetylcholine benzilate (50 μM) to mousebrain acetylcholine receptors from rat brain with an IC50 of 20 μM. In conclusion, the reaction of ATX with the nicotinic receptor appears remarkably similar to that of ACh. Since ATX does not change the properties of the endplate, it is quite possible that the partial agonist effect of ATX is due to the hydroxylation of the motor nerve that plays a major role in the ultimate transmitter action. Thus, ATX promises to serve as a most important tool for the study of the nicotinic receptor. (Supported by NIH Grant NS-19363.)


An attempt to understand the role of nerve fibers on the development of skeletal muscle and on its acetylcholinesensitivity is being made by examining the superior oblique muscle of the duck embryo in which contact by the trochlear nerve is experimentally prevented. Trochlear nerve fibers normally reach the muscle on day 10 of incubation and the muscle at this time is primarily composed of myoblasts and a few myocytes. In order to destroy the trochlear nucleus dorsal midbrain lesions were made on day 7 of incubation by using electrocautery. The absence of the trochlear neuromuscular junctions at this time was confirmed by histochemical methods. Lanthanum (La++) in micromolar concentrations has been shown to antagonize irreversibly evoked transmitter release by blocking Ca(++) entry into nerve endings. Such an effect has been confirmed in this study. In addition, however, a small elevation in the extracellular Ca(++) concentration in the presence of La caused an immediate, forceful increase in evoked transmitter release. It can be demonstrated that this effect of Ca(++) is not due to displacement of La(3+) by Ca(++) or to the effect of Ca(++) on evoked transmitter release. These results suggest that the effect of Ca(++) on evoked transmitter release is not mediated by a direct interaction between the two cations. The simplest explanation appears to be that increasing the external Ca(++) concentration surmounts the La(3+) antagonism by 'mopping-up' small external receptor sites. This interpretation is consistent with the observation that the following should hold: a) the partial agonist, Sr(2+) (which must occupy a large fraction of the binding sites of the receptor sites) could be antagonized by La(3+) and an increase in the external Ca(++) concentration should not restore transmitter release; b) comparison of equal levels of transmitter release by Ca(++) and Sr(2+) should provide an affinity constant for Sr(2+) that is similar to the value determined for Sr(2+) as an antagonist of Ca(++)-mediated release. The observed behaviour is in accord with these predictions. These results suggest that certain activities of La(3+) exist at frog motor nerve terminals and demonstrate that the mathematical approach of modern drug receptor theory is more appropriate than HMM kinetics when analyzing the agonist effects of divalent cations on transmitter release.

ANTICHOLINERGIC EFFECT OF AMANTADINE IN MOTOR NERVE TERMINALS. F.C. Su, M.D., V.D. Hensley, PhD* and A.H. Posner, M.D. Division of Neurology, SUNY at Stony Brook, N.Y., Dept of Histology, Columbia University, N.Y.

Amantadine is an effective antiparkinsonism agent especially for the symptom of tremor and rigidity. The mode of action has been suggested that Amantadine causes release of dopamine (Greath, et al. Science 159:203, 1970). Due to loss of dopaminergic neuron in parkinsonism such a mechanism probably plays little role. We have, hence studied the effect of Amantadine on cholinergic nerve terminals and our results indicate that Amantadine posses significant anticholinergic effects in motor nerve terminals. We used in vitro mouse phrenic nerve-diaphragm preparations. Intracellular recording of network potentials (n+); endplate potentials (EP) and miniature endplate potentials (MEPP) were made by glass microeleetrode filled with 3M KCl. The EP's were obtained by nerve stimulation in high magnesium Kreb's solution or in glycerol treated muscles. At least 100 to 150 EP's were recorded on 64 channel along with 60 to 100 MEPP's in each muscle fiber. Offline analysis using an IMP 360/44 was used. The analysis includes the quantal content, probability of release (Q) and available store (n). The quantal content were determined by direct n methods using the ratio of mean amplitude of EP's and MEPP's after correlation of non-linear response of EP. The n and Q were determined assuming binomial distribution of quantal release and according to formula given by Johnson and Kemig (J. Physiol. 218:757, 1971).

Amantadine at 50 μM and 150μM concentration significantly reduces the amplitude of EP's in motor nerve terminals. This effect is dose and concentration dependent. The endplate potentials were depolarized from -78 mV to -58 mV at 150 μM concentration and the depolarization was not noticeable at 50 μM concentration. Quantal content decreases at 11% showed a 5% to 65% reduction at 150 μM and a 1% to 20% reduction at 50 μM. No change in the n and Q were noted. The effect on quantal content has also been studied, which usually takes 60 minutes, where the presence of MEPP and MEPP's is rapid and complete within 10 minutes. The time course of reduction in quantal content is quite similar to muscle membrane depolarization. The time course of the reduction that result might be caused by the depolarization of presynaptic nerve terminals. Based on our findings and others (Nastuk, et al., Nature 264:76, 1976), we propose that anticholinergic effect of Amantadine both presynaptically and postsynaptically accounts for its action in antiparkinsonism.

Supported by Veterans Administration General Research Fund 8206-01.

Antibodies directed to specific components of motor nerve terminals could be used to study molecular processes in presynaptic function, such as transmitter release, at the neuromuscular junction. Recent success in obtaining purified cholinergic synaptic vesicles from the electric organ of Torpedo californica (Carlisle et al., 1978) has enabled us to raise anti-synaptic vesicle antibodies in rabbits, and to demonstrate by indirect immunocytochemical techniques that antibodies in rabbit anti-synaptic Vesicle antibody (RSAV) bind to nerve terminals in rat, chick and frog skeletal muscle. Cryostat sections were incubated with a mixture of RSAV and rhodamine-conjugated antithrombiculin (α-BTx). Appropriate filter combinations allowed us to excite and view the fluorescein or rhodamine fluorescence, and thus to compare the distribution of the immunohistochemical stain with that of α-BTx, which binds tightly and specifically to the acetylcholine receptors that are the molecular units of the neuromuscular junction. Antibodies stained a variety of membranes, but most intensely stained were small patches on the muscle fiber surfaces, which could be identified as neuromuscular junctions because they also stained with rhodamine α-BTx. The RSAV-stained structures were identified as nerve terminals on the basis of their size, shape and position, and because they were absent from denervated muscle. After adsorption of RSAV with vesicle-free membranes from Narcine electric organ, nerve terminals stained intensely, but some internal nerve or in intramuscular nerve branches did not stain well, nor did the cytoplasm of motor neuron somata. Thus, antiserum distinguishes terminal from preterminal portions of motor axons. In particular, the antigen antibodies in RSAV bind to nerve terminals in frog skeletal muscle, for it is in frog muscle that the most detailed evidence has been obtained for the vesicular hypothesis of transmitter release. This hypothesis predicts that antibodies on vesicles should be hidden from antibodies applied to whole muscle, but exposed when vesicles fuse with the plasma membrane during stimulation.

This work was supported by a grant from NIH (NS09978) to RKK. JRS and ECG are fellows of the Muscular Dystrophy Association. RJW is a predoctoral fellow of the NIH.

1665 IMMUNOHISTOCHEMICAL LOCALIZATION OF ACETYLCHOLINE RECEPTORS ON RAT SKELETAL MUSCLE SURFACE WITH MONOCLONAL ANTIBODIES. Riley C. Yu, D.P. Richman* and C.M. Gomez*, Department of Neurology and Brain Research Institute, University of Chicago, Chicago, IL 60637.

Progress towards understanding neural organization and conduction at the neuromuscular junction has been greatly aided by studies of binding properties of cholinergic ligands to membrane receptors using biochemical and morphological techniques. We have used anti-acetylcholine receptor (AChR) antibodies secreted by cloned hybridomas produced by fusion of a myeloma cell line with spleen cells from animals immunized with acetylcholine receptor (AChR) antibodies secreted by cloned hybridomas produced by fusion of a myeloma cell line with spleen cells from animals immunized with purified Torpedo electric organ antibodies (Castellani et al., 1978, J. Biophys. Res. Comm., 1979, in press). Dissociated cells from leg muscle of newborn Wistar rats were first preplated for myoblast enrichment before being seeded on culture plates coated with growth factors and cultured with normal medium. AChR appeared on the membrane surface as the myoblasts fused to multinucleated myotubes. Thus, antiserum distinguishes terminal from preterminal portions of motor axons. It is in particular fortunate that antibodies in RSAV bind to nerve terminals in frog skeletal muscle, for it is in frog muscle that the best evidence has been obtained for the vesicular hypothesis of transmitter release.

RJW is a predoctoral fellow of the NIH.


There are a number of differences in the literature concerning the kinetic properties of facilitation of transmitter release. Pallant & Hartin (1967) and Magleby (1973) were able to account for the increase in transmitter release during short trains of repetitive stimulation at the frog neuromuscular junction using a linear model of facilitation. Younkin (1974), on the other hand, developed a two-step kinetic model to describe transmitter release in the frog. More recently, Balnave & Gage (1977) were unable to describe data they obtained from the toad neuromuscular junction with a linear facilitation model, but used instead a two-step kinetic model. In all of these previous studies the data were analyzed without correcting for changes in transmitter release that might arise from augmentation and potentiation, two processes with time constants longer than facilitation that also act to increase transmitter release during and following repetitive stimulation. We have examined several models of facilitation, taking into account the effects of augmentation and potentiation.

End-plate potentials (EPPs) were recorded from the frog sartorius nerve-muscle preparation under conditions of low quantal content (0.4-0.6 Miller units of a 490 Hz). The nerve was conditioned with short trains of impulses (20-40 impulses at 10-100/sec), then tested with single impulses applied at varying intervals after the conditioning stimulation. The decay of the testing EPP amplitudes was corrected for the contributions of augmentation and potentiation and estimates of facilitation were obtained assuming three models: a linear model, such that

\[ F = (F^*1 + 1)(F^*2 + 1) \]

where \( F \) is facilitation and \( F^*1 \) and \( F^*2 \) are the factors responsible for the first and second components of facilitation; a power model, such that

\[ F = (F^*1 + 1)^{F^*2} \]

where \( q \) is the power relating the factors responsible for facilitation to facilitation; and a multiplicative model, such that

\[ F = (F^*1 + 1)(F^*2 + 1) \]

Both the power and multiplicative models of facilitation could describe the effect of repetitive stimulation on facilitation of transmitter release; the additive model consistently gave poorer fits to the data. Supported by NIH grant NS 10277.
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the slow outward current must be hyperpolarized relative to the inward and outward currents remain partially activated throughout the entire portion of the depolarizing phase of the oscillation; in model are analogous to movement along paths (trajectories) of motion requires an NRC, which must persist throughout the acceleration process. Such trajectories influence the frequency and amplitude of the oscillations in both types of neurons. Most experiments were performed on the follower cells, which are larger and consequently more readily examined with intracellular electrodes. Bath application of octopamine (OCT), dopamine (DA), epinephrine (EPI) or norepinephrine (NE) produced increases in the frequency of bursting activity of follower cells, OCT was the most effective, increasing burst frequency by 120% (± 10 S.E.M.) at a concentration of 10^-7 M. The excitatory effects of all four amines was reduced by the α-adrenergic antagonist phentolamine (10^-6 M). Changes in follower cell burst frequency were a consequence of corresponding changes in current activity. Recordings from pacemaker neurons during amine treatment showed that the frequency of spontaneous action potentials increased, accompanied by a small (less than 1 mV) depolarization of the membrane potential. A transient decrease in pacemaker action potential rate was observed with DA or NE perfusion. In addition to their excitatory effect on follower cells, amines also directly influence follower cell activity, DA and NE produced transient hyperpolarizations of follower cell membrane potential (up to 20 mV at 10^-7 M), and OCT depolarized these cells (less than 10 mV at 10^-6 M). These effects are not due to alterations in the synaptic input on to these cells, for they occurred in preparations where synaptic activity was blocked by addition of Co ++ or Mg ++ , or removal of Ca ++ . Thus these biogenic amines are active on both types of neurons which comprise the Limulus cardiac ganglion.

Certain features are common among neurons which display an intrinsic slow oscillation of membrane potential: a steady-state inward current associated with a negative slope conductance (INH), and a depolarization-induced slow outward current (Sci. 186:232 (74)). However, the exact circumstances under which oscillations will arise have remained unclear; for example, not all cells with an INH oscillate, and some question has even been raised as to whether all INH cells are oscillatory. (Sci. Rep. 94:161 (75), Neuro-Sci., 565 (74)). Detailed models of oscillating neurons, while elegant, are cumbersome and have tended to obscure critical features of the interaction between these membrane current components. To delineate the conditions which must be met in order for oscillations to occur, and to determine the factors which underlie distinguishing characteristics of the oscillations, a model which incorporates the slow outward and INH-associated inward current was studied.

This model led to a set of non-linear differential equations which describe a phase space in which changes of the state of the model are analogous to movement along paths (trajectories) of "rapid" or "slow" motion (Andronow et al., Theory of Oscillators, p. 660 (Pergamon, 1966)). Piecewise linear analysis of these equations, confirmed by analog computer simulation, demonstrated several points clearly, including the following: 1) The oscillation requires a multiple period depolarizing portion of the depolarizing phase of the oscillation; 2) increased leak conductance can abolish the oscillation; 3) the inward and outward currents remain partially activated throughout the oscillatory cycle; 4) a too rapidly activating outward current can prevent oscillations; 5) the reversal potential of the slow outward current must be below the INH region, or else inactivation of the inward current must play a dominant role in the repolarizing phase of the oscillation. This, in turn, also depends on the participation in the output regulation (i.e. how the frequency and amplitude of the oscillation depend on variations in the control parameters such as the baseline firing rate of the oscillations in the more general non-linear model and in the real cell.

In the presence of D-glutamate and curare the isolated cords were capable of generating effenter activity having all the characteristic features of natural waves: 1) There was strict alternation between two roots of a single segment. 2) The duration of a burst of a VR increased linearly with the cycle duration, as is the case in intact animals. The latency of the effenter roots increased linearly with cycle duration, i.e. there was constant phase coupling between the segments. To date, a piece of cord consisting of as few as 6 segments could produce each pattern, although such reduced pieces may be unstable. The fibers ensuring the phase coupling were found to be highly distributed, as well illustrated by current injection which caused a ringing after extensive transverse lesions including 80% of the cord width. The location of the lesion had effects on the coupling, but no region was found which was effective for the establishment of either a coupling or an uncoupling. Many different regions could affect the structure of the cycle. In addition, the alternation between the roots of a single segment was not disrupted by longitudinal lesion of 1-5 segments in the midline region.

This is the first preparation in which a complete locomotor pattern has been present in a in vitro preparation of the central nervous system maintained under in vitro conditions. Moreover, the effenter pattern can be highly stable and show all the essential features of the normal pattern.


Little is known of the cellular organization of vertebrate central pattern generators (CPG's). Swimming in fish is a complex motor behavior which is controlled by CPG's in the spinal cord (Griller et al., Brain Res., 109:255-269, 1976). The undulatory body movements of fish result from a specific activation pattern of a group of locomotor muscle fibers. The two sides of the body are made up of single segment and a constant phase coupling between successive segments (Griller, Exp. Brain Res., 20:145-147, 1974). The detailed models of pattern generation for locomotion in detail, we have used an in vitro preparation consisting of the isolated spinal cord of Tetraodon nigroviridis. 50-60 segments were dissected out along with the motores and surrounding membranes. The tissue was maintained in cooled aerated lamprey Ringer (ph = 7.4). Curare was added to prevent the small amplitude movements sometimes produced by the residual muscle tissue. Low concentrations of D-glutamate or 1-DOPA were used to induce swimming movements, following the technique of Ploem in the lamprey "actinone" preparation (submitted and cf. Teräväinen and Rovainen, J. Neurophysiol., 34:990-998, 1971). The ventral root (VR) discharges were monitored with suction electrodes in the conventional manner.

In the presence of D-glutamate and curare the isolated cords were capable of generating effenter activity having all the characteristic features of natural waves: 1) There was strict alternation between two roots of a single segment. 2) The duration of a burst of a VR increased linearly with the cycle duration, as is the case in intact animals. The latency of the effenter roots increased linearly with cycle duration, i.e. there was constant phase coupling between the segments. To date, a piece of cord consisting of as few as 6 segments could produce each pattern, although such reduced pieces may be unstable. The fibers ensuring the phase coupling were found to be highly distributed, as well illustrated by current injection which caused a ringing after extensive transverse lesions including 80% of the cord width. The location of the lesion had effects on the coupling, but no region was found which was effective for the establishment of either a coupling or an uncoupling. Many different regions could affect the structure of the cycle. In addition, the alternation between the roots of a single segment was not disrupted by longitudinal lesion of 1-5 segments in the midline region.

This is the first preparation in which a complete locomotor pattern has been present in a in vitro preparation of the central nervous system maintained under in vitro conditions. Moreover, the effenter pattern can be highly stable and show all the essential features of the normal pattern.

NEURONAL CIRCUITS AND PATTERN GENERATION

Each fiber of the fibrillar flight muscles of Drosophila melanogaster is innervated by a single motor neuron. Recordings from individual muscle fibers have revealed that motor neurons innervating different fibers of a segment tested for a large discharge or a burst, with each motor neuron firing once per cycle. Of the six possible firing sequences for the four motor neurons innervating the ventral, lateral, and dorsal (uniaxial c. d.) muscles (sequences 1-4, 4, 2-4, d-e, f, are preferred. Subsequent to antidromic stimulation of one motor neuron, firing in the others is inhibited for tens of milliseconds and the cycle is shifted. To account for these results Harcombe and Wyman (J. Neurophysiol. 40:1066-1077, 1977) proposed that the motor neurons innervating the latter form an intrinsic circuit in which each motor neuron innervates each of the others and that this mutual inhibition is greatest between c·d and between e-f. To determine whether this circuit can account for motor neuron firing patterns observed during Drosophila flight, we have constructed an equivalent circuit with electronic neural analogues (neuromimics). These neuromimics were designed by Lewis (Proc. Inst. Elect. Engrs. 56:931-949, 1968) and have been employed in previous modeling studies of neural networks (Friesen and Stent, Biol. Cybernetics 28:1-24, 1977). The Lewis neuromimics mimic the Hodgkin-Huxley parameters describing large-scale voltage fluctuations at the neural impulse initiating zone and can model both excitatory and inhibitory synaptic interactions. We have provided our neuromimics with common excitatory input and adjusted them so that each had a 6 Hz free-running impulse frequency. We then connected each neuromime to each of their inhibitory inputs, setting synaptic time constants to 30 ms and synaptic amplitudes such that inhibition between those neuromimics modeling muscles of one segment of Drosophila and Homarus is the same each time they occur but can have extra spikes superimposed on the pattern. These extra spikes could be due to background noise or a variation in the pattern. Our new pattern detection method tests whether a temporal pattern occurs more than expected in a null model. The random model used is a shuffled version of the data spike train. In each comparison, extra spikes in the template or in the spike train are considered 'significant' if each occurs more than expected at random. The test is achieved by comparing each template to every place in the spike train to every place in a number of shuffled versions of the spike train. In each comparison, extra spikes in the template or in the spike train are considered 'significant'. Those patterns which match the spike train in a statistically more than random number of times are considered significant. Supported by grants NS05666, NIH-M012799, BR0896-05415.


Repeating patterns in nerve spike trains could be important for information transfer in the nervous system. Patterns could be generated in the brain to drive certain behaviors at times unknown to the experimenter. Thus, it is appropriate to search for patterns of firing which occur frequently but at unspecified times in the spike train. Such patterns might not be evident in an autocorrelogram or with some other spike train method. We have developed a new pattern detection method based on comparing a series of template patterns to the spike train. This method differs from our previously presented method (Dayhoff and Gerstein, Neurosci. Abst. 3:300, 1978) because it is able to detect frequently occurring spike patterns which are approximately the same each time they occur but can have extra spikes superimposed on the pattern. These extra spikes could be due to background noise or a variation in the pattern. Our new pattern detection method tests whether a temporal pattern occurs more than expected in a random model. The random model used is a shuffled version of the data spike train. In each comparison, extra spikes in the template or in the spike train are considered 'significant' if each occurs more than expected at random. The test is achieved by comparing each template to every place in the data spike train to every place in a number of shuffled versions of the spike train. In each comparison, extra spikes in the template or in the spike train are considered 'significant'. Those patterns which match the spike train in a statistically more than random number of times are considered significant. Supported by grants NS05666, NIH-M012799, BR0896-05415.


The latency of the acoustic startle reflex in the rat is 6-7 msec., measured from tone onset to the beginning of the electromyographic response in the forepaw (Ison et al., Physiol. Behav., 1973, 10, 1035-1039). This extremely short latency indicates that only a few synapses could be involved in the primary acoustic startle circuit. Acoustic startle is being used as a model system for studying habituation, sensitization, presynaptic inhibition, classical conditioning, fear or anxiety, and drug effects on behavior. Hence, the delineation of the exact acoustic startle circuit would provide critical information for further study in all of these areas. Because of the ease of investigation, the possibl neural connections between the cochlear n., which receives the primary afferent input, and the reticulo-spinal tract, which most probably represents the final motor pathway of the startle circuit. Unilateral, single pulse electrical stimulation of specific ventral points within the n. reticularis pontis caudalis, the cell bodies of the reticulo-spinal tract, elicits short latency, startle-like responses in unanesthetized rats. Bilateral lesions of these points abolish acoustically elicited startle responses. Reaction product from horseradish peroxidase dionized into these points is found in the n. reticularis pontis caudalis, which is known to be innervated by the cochlear nucley. Single or multiple-pulse stimulation of the n. reticularis pontis caudalis eicit short latency startle-like responses. Bilateral lesions of this n. attenuate or abolish acoustically elicited startle. Latencies of electrically elicited startle become shorter as electrodes are placed progressively further down the circuit. Simultaneous stimulation of electrical and acoustic stimuli elicit startle amplitudes greater than the sum of each presented alone. Temporal interactions between acoustic and electrical stimuli occur which are absent when the stimuli are given in sequence. The data suggest that the primary acoustic startle circuit in the rat is: auditory nerve, cochlear nucleus, n. reticularis pontis caudalis, reticulo-spinal tract, n. reticularis pontis caudalis, interneuron, lower motor neuron, muscles. Hence five synapses, plus the neuromuscular junction, are probably involved.

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PARALLEL RECORDING OF SINGLE UNIT ACTIVITY IN VIVO.

1675 NEURONAL BASIS OF THE SWIMMING RHYTHM IN TRITONIA.
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Department of Biological Sciences, Stanford University, Stanford, CA 94305.

The swimming rhythm of the marine mollusc, Tritonia diomedea, is a series of 2-20 cycles of alternating dorsal and ventral flexions. The central pattern generator mechanism controlling the swimming consists of at least three populations of cerebral premotor interneurons designated C2, dorsal swim interneurons (DSI) and ventral swim interneurons (VSI). The temporal firing pattern of these interneurons during a swim sequence was analyzed to elucidate neuronal mechanisms involved in the initiation, maintenance, and termination of the swim pattern. Over the time course of a swim sequence, cycle period progressively increases. For a given preparation, oscillation terminates when the cycle period attains a maximal duration independent of the number of preceding cycles. Each swim cycle may be subdivided into three segments by the firing times of the three interneurons populations. Variation in the initial segment of each cycle accounts for 80 percent of the total increase in cycle period. The latter two segments remain relatively constant independent of cycle period. Cyclical bursting within the swim network is superimposed on a large average depolarization (10-15 millivolts) which slowly dissipates over the course of a swim sequence. Oscillation rate appears to be directly proportional to the level of tonic network excitation (e.g. cycle period decreases as tonic excitation increases).

Swim network oscillation is initiated by a tonic depolarization established by a transient sensory stimulus. Pattern generation is maintained as long as phasic positive feedback between C2 and the DSI is established during the initial and middle segments of each swim cycle. Positive feedback between C2 and DSI is required for maintenance of oscillation but is not required for the generation of a swim cycle. During a swim sequence, dissipation of the tonic depolarization level decreases the likelihood of the positive feedback. Oscillation terminates when the tonic network excitation fails to provide sufficient CI/DSI positive feedback. A swim sequence ends on a weak dorsal swim interneuron burst corresponding to the last dorsal flexion of the behavior.

1676 RHYTHMICALLY OCCURRING SYNAPTIC POTENTIALS IN TRIGEMINAL MOTONEURONS EVOKED BY REPEATED CORTICAL STIMULATION L. J. Goldberg and M. Tal.

Chewing, licking and sucking are motor behaviors which have a strong rhythmic component. The guinea pig is used in this study as an experimental model. The trigeminal nervous system pattern generators which may be responsible for the production of these rhythmically occurring jaw movements. A single shock or short train of shocks delivered to the motor face area of the cortex of ketamine anesthetized guinea pigs evokes a brief jaw opening response. Intracellular recordings in trigeminal motoneurons innervating the jaw opener and closer muscles reveal that this stimulus evokes membrane depolarization and spikes in jaw opener, and a reciprocal hyperpolarization in jaw closer motoneurons. The latency from the cortical stimulus to the onset of the response is 10 msec and the duration 20 msec. The hyperpolarization in jaw closer motoneurons changes to a depolarizing potential with the injection of Cl- and can be reversed by hyperpolarization of the membrane with current passed through the recording microelectrode. These results indicate that the hyperpolarization is postsynaptic. When the stimulus intensity is reduced to well below threshold to evoke a response to a single shock, continuous stimulation at frequencies of between 20 and 40 Hz produces the following: the initial stimuli of the train do not evoke a response in the motoneurons; after a variable period of time, each succeeding stimulus gradually becomes less effective, the opening of the closer motoneuron spiking ceases, the hyperpolarization in the closer motoneurons diminishes, and the jaw returns to the original rest position. Stimulation of the cortex at a frequency of >2 Hz and opening of the jaw is produced. Cortical stimulation with the animal paralyzed evokes a similar pattern of synaptic response. The results suggest that, 1) a facilitatory mechanism is activated by the high frequency cortical stimulation which greatly increases the effectiveness of the motor cortex-trigeminal motoneuron pathway; 2) a centrally mediated oscillation can be activated which rhythmically gates this facilitation of the pathway. These central mechanisms result in rhythmic jaw movements similar in behavioral form to those observed in chewing or licking behavior. Supported by NIH Grant DE 4166.

1677 ORGANIZATION AND GENERATION OF PREURYNEURON BURSTS DURING TRITONIA SWIMMING.

Escape swimming in Tritonia diomedea is characterized by alternating bursts of activity in two populations of antagonistic motor neurons, the Dorsal Flexion Neurons (DFN) and the Ventral Flexion Neurons (VFN). The basic swim pattern is generated by an identified network of premotor interneurons and identified flexion neurons. The VFN pool is relatively homogeneous. Swim bursts in VFNs are 1.5-3 seconds in duration and begin at virtually the same time in all VFNs. The DFN pool consists of neurons with a large range of parameters. The DFN-A bursts are of long duration (3-5 seconds), have two frequency peaks per burst, and increase in duration as cycle time increases. The latency from the beginning of a DFN-A burst increases as cycle time increases, such that DFN-A bursts occur at nearly a constant phase in every cycle. The DFN-B bursts are of shorter duration (2-3.5 seconds), have a single frequency peak, and do not increase in duration as cycle time increases. The latency from the beginning of a DFN-B burst to the onset of a DFN-B burst increases as cycle time increases, such that DFN-B bursts occur at nearly a constant phase in every cycle. Recordings from whole animal preparations indicate that cells in the DPN-B pool are responsible for generating most of the dorsal flexion movement. Simultaneous intracellular recordings were made from identified pattern generator interneurons (C2, DSI, VSI) and identified flexion neurons in a search for monosynaptic connections. Monosynaptic sensitivity was judged by 1) postsynaptic potentiation (PSP) at constant latency following a presynaptic spike 2) ability to affect postsynaptic potentials of the presynaptic pool and 3) maintenance of postsynaptic potential in 2 1/2 times normal Ca. The synaptic connections from the interneurons to the two classes of flexion neurons are very different. The C2 excites both classes of flexion neurons, whereas the DSI excites only the DFN-A cells but inhibits DFN-B cells. No monosynaptic connections were observed from flexion neurons to interneurons, nor among flexion neurons. The identified connections from C2, DSI and VSI appear to be sufficient to account for much of the temporal organization of flexion neuron activity during a swim.

1678 PARALLEL RECORDING OF SINGLE UNIT ACTIVITY IN VIVO.

We have developed a high-density fixed-array multi-electrode system for monitoring extracellular neural activity in vivo. The PRONG (Parallel Recording of Neural Groups) can be used to simultaneously record from two or more neurons at a depth of 2-3 mm in the brain tissue that is accessible within 7 mm of the surface. It is fabricated using microelectronic technology and each recording site is 100 μm in diameter with a rise time of >50 μsec, and is postsynaptic. The leads start at contact pads at the top of the PRONG and end in a wedge-shaped area containing an array of 20 recording sites (100 μm spacing) at the bottom tip of the PRONG. The shunt capacitance is about 3 pf. The area of the exposed recording sites can be varied during fabrication from 40 to 120 μm², and each site is plated with platinum black. For implantation, the PRONG is supported by a connector fixed to a microdrive. The connector spring-loads each of the contact pads of the PRONG with thin beryllium copper wire. The PRONG has been used to record single units in cat visual cortex with 250 μv amplitude spikes with a signal to noise ratio of 10:1.

The new versions of the PRONG with many recording sites and a thinner active area are under construction. (Research supported by NIH grants 5 RO1 EY00676 and 1 T31 HD07444.)

[Graph showing recording sites and data from PRONG implantation.]

340 μm 10 μs

100 μm 10 μs
The activity of single cells was recorded chronically in awake freely moving animals from the centromedian-parafascicular (CM-Pf) area of the cat thalamus using fine wire semimicroelectrode bundles. Neuronal spike trains were analyzed by a pattern detection model which made no assumptions about consecutive interspike intervals; an interval was either longer (+) or shorter (-) than the previous interval. Thus a consecutive series of (+) and (-) signs was generated which corresponded to the interval lengths of the neuronal spike train. A movable window of variable size then moved through the spike train one sign at a time tabulating occurrences of patterns 3 through 6 signs in length. The number of occurrences of each pattern encountered was compared to the expected occurrence calculated from a theoretical model which assumes independence (Bridgus and Marczyński Brain Res. 125: 65, 1977). Chi-squared tests of significance were used for all comparisons. Patterns which were emitted more often than expected from the theoretical model and those which were suppressed, i.e. appeared significantly less often than predicted, were both seen in the data and often co-occurred in an ensemble, or cosmographic profile of patterns for a particular behavioral state.

Many neurons were found which, in paired sets of data, showed dimetric tendencies; patterns emitted in behavioral states were significantly suppressed in another and vice versa. Several neurons showed this type of behavior during slow wave sleep (SWS) and quiet wakefulness; other neurons were diametrically opposed during motor behavior. Some of the patterns in an ensemble did not discriminate cases only one pattern would reverse. In many instances diametric shifts would occur without changes in the mean firing rate. 

The results suggest that neurons modulate the signal-to-noise ratio in certain pathways by actively suppressing certain temporal patterns below their chance occurrences. Even a small dimetric shift in these patterns would constitute a highly significant signal against an otherwise noisy background. The observed neuronal behavior is comparable to an anacoluthon, a change in syntax to achieve rhetorical effect, hence we propose to term this neuronal behavior anacoluthic.

Electron microscopy showed cytoplasmic vacuolization, and connectivity with a new technique for rapidly killing all or part of single identified neurons. To examine the effect of removing one cell from a circuit, the tecterotectic synapses were impaled with two electrodes. In the E IV ganglion, two motor patterns, and the connectivity of the 30 neurons it contains has been worked out. The first cell was filled with the dye Lucifer Yellow CH via intracellular iontophoresis. The dye itself had no effect on the resting-, and action-potentials. The Lucifer Yellow CH is an indifferent dye, not toxic to the cell, and does not affect the neuronal resting-potential. 

Firing pattern I consists of trains of three to ten evenly spaced action potentials. Interspike intervals within a train range from 200 to 400 msec, and trains are separated by 2 to 10 seconds. Hyperpolarization of the cell reveals a pattern of inhibitory postsynaptic potentials (IPSPs). The IPSPs induced on stimulating the PN area could be antidromically activated. The presence of this response was further studied by systematic tracking with the stimulating microelectrode in sagittal (L.O.S) and transverse (P.7.5) planes through the ipsilateral BIN area. There were two or three low threshold foci (less than 30uA) which were separated by nonresponsive sites. These foci were found mostly in the BIN area (around 0.5 mm lateral from the midline, and 1.0 to 5.0 mm deep from the floor of the fourth ventricle), indicating axonal branching in this area. 

During intracellular recording of BINS, microstimulation of the PN area induced a hyperpolarization with latencies ranging from 0.7 to 1.1 msec, and at least two IPSPs. These IPSPs were either depolarizing potentials by passing hyperpolarizing currents. This indicates that the hyperpolarizations are monosynaptic IPSPs. The IPSPs induced on stimulating the PN area could explain IPSPs found in the same BINS during the slow wave of nystagmus. 

It is concluded that burst activity of BINS may be caused by release of the direct inhibition from MNs (disinhibition).
MODELLING OF GANGLION CELLS IN A REALISTIC VERTEBRATE CONE AUTORADIOGRAPHIC

M.K. Sanghera, D.C. German, M. Mendershausen, and R.S. Kiser.

The mesencephalic central gray area (CG) is not a functionally homogeneous structure: for example, the dorsal CG component of the central gray produces aversive behavioral effects while the ventral portion, which contains the serotoninergic dorsal raphe nucleus, has an沮impulsive gadgetology.

Electrical stimulation of the lateral central gray has been shown to induce the lordosis reflex in the female rat, and luteinizing hormone releasing hormone terminals in the hypothalamus have been observed. It has been speculated that these terminals may be involved in the lordosis reflex. Intra-central gray projections go to the pretectal area. Labelled fascicles were seen to course ventromedially in discrete fascicles on the lateral edge of the central gray. Rostrocaudally projecting fascicles coursed through the ventral tegmental area and the medial forebrain bundle. Labelled fibres radiated rostrally and ventrally in Weisschedel's radiatio grisea.

The mesencephalic central gray area (CG) is not a functionally homogeneous structure: for example, the dorsal CG (1) and ventral CG (2) are different areas. From the dorsal CG injection site, extensive bilateral projections were made into the (1) dorsal CG (2) ventral CG and (3) lateral CG. Iontophoretic injections of 35S-methionine were made into these areas. Rats survived for 3 days. Following a 6 day exposure period, the tissue was developed, stained for Nissl and examined microscopically. From the dorsal CG injection site, extensive bilateral labelling was observed. The commissure of the superior colliculus was labelled both rostrally and caudally. Labelled fibres radiated laterally and ventrally in Weisschedel's radiatio grisea.

Intra-central gray projections went to the pretectal area and periventricular nucleus of the thalamus. From the lateral CG, three rostrally projecting pathways could be identified. Heavy labelling was observed in the commissure of the superior colliculus from the injection site to the pretectal area. Labelled fascicles were also seen to course ventromedially in discrete fascicles on the lateral edge of the central gray. Rostrocaudally projecting fascicles coursed through the ventral tegmental area and the medial forebrain bundle. Labelled fibres were seen as far rostral as the preoptic area. Finally, in the thalamus, these diverse projections from different parts of the CG may underlie the functionally different areas associated with this structure. (Research supported by BRS grants 5-807-RR07175-03 and 5-807-RR05426-16).

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Cardiac ganglia of arthropods are favorable preparations for determining how neurotransmitters or neurohormones modulate neuronal activity. We have addressed this question by using the cardiac ganglion and heart of the horseshoe crab, Limulus polyphemus. Little is known about the regulation of cardiac ganglion activity in vivo; we therefore monitored the heartbeat and cardio regulatory nerve activity of intact Limulus using chronically implanted electrodes. The heartbeat provides a direct indication of cardiac ganglion activity, since it is generated by this ganglion. The Limulus heartbeat is altered in a variety of situations. Decreases in rate are associated with increases in the electrical activity of efferent units recorded extracellularly from the cardio regulatory nerves connecting the CNS and cardiac ganglion. There are other efferent units whose activity is correlated with increases in heart rate. This suggests that the CNS contains both excitatory and inhibitory cardio regulatory neurons. We have attempted to determine the neurotransmitters or neurohormones involved in cardio regulation. As amines are thought to serve a cardio regulatory role in other systems, we examined the catecholamine levels of the cardiac ganglion, cardio regulatory nerves, and CNS. Dopamine, norepinephrine, and epinephrine were found in all three of these structures using high pressure liquid chromatography with electrochemical detection. Octopamine has also been found in the cardiac ganglion by others. The presence of biogenic amines in these structures has been verified by localization with a glyoxylic acid histofluorescence technique. All four amines excite the isolated ganglion, increasing the rate of bursts of electrical activity recorded extracellularly. In summary, the activity of the cardiac ganglion of Limulus is modulated in vivo by neurons within the CNS. Biochemical, histoch emical, and physiological data suggest that one or more amines may be involved in this cardio regulation.

Supported by the Grass Foundation, NSF, NIH, WHO, and Chesapeake Bay Research Funds.

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**A QUANTAL ELECTRIC CONNECTION BETWEEN NEURONS IN THE LEECH CNS.** Birgit Zipser, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, U.S.A.

A synaptic connection exists between leech neurons that is quantal in nature but is not blocked under conditions which interfere with chemical synaptic transmission. The connection is important for the neural control of mating since it occurs between two pairs of penile evertor motor neurons, the lateral and rostral cells. These neurons have previously been found to be directly coupled via an electrotonic synapse. In addition to this electrotonic transmission, I have found another excitatory lateral-to-rostral interaction with unusual properties. Transmission across this synaptic connection is unidirectional with a latency of about 4 msec. Intermittent failures in transmission are seen. These failures are not due to conduction block in the lateral cell since lateral cell spikes invariably give rise to small electrotonic coupling potentials in the rostral cell. The amplitude of the postsynaptic potentials (PSPs) fall into two discrete classes, with the larger PSPs being about twice the size of the smaller ones. In some preparations, spontaneous PSPs occur; these have amplitude distributions similar to evoked PSPs. These findings would ordinarily suggest that the interconnection is chemical; however, ion substitution experiments rule out a conventional chemical synapse. The PSPs persist in Ca-free solutions, including those to which the Ca-chelator EGTA (0.5 to 1mM) has been added. The single and double modes of the PSP amplitudes are not only maintained in Ca-free Ringer, but in fact, the number of failures decreases. Increasing the external Ca concentration inhibits transmission by increasing the number of failures. Ca channel blockers such as Ni (2.5mM) do not block transmission either. The mechanism by which this Ca-independent quantal transmission occurs is not clear. One way that the transmission could proceed is via the presence of 2 interposed interneurons with parallel electric connections to both rostral and lateral cells. The single and double PSPs could be generated by action potentials in one or both of these neurons. The facilitatory effects of Ca deletion could then be attributed to removal of chemical inhibition from these hypothetical interneurons. Consistent with this hypothesis is the finding that the PSPs are blocked by tetrodotoxin at concentrations that do not eliminate action potentials in lateral and rostral neurons. Supported by NSF grant 78-13064.
NEURONAL SHAPE AND FUNCTION

Sections from the caudate nuclei were illuminated with a 325 nm He-Cd laser and studied with a tricorocular microscope modified with a dichroic mirror and quarter optic. Tissue fluorescence could either be photographed or measured by a monochrometer and photon counting system. 325 nm laser illumination of caudate sections revealed a number of endogenous fluorescent structures of potential neuroanatomical importance. These included: long fibers with bright blue fluorescence, triangular “nerve cell-like” structures and unresolved areas of fluorescence. The long fibers contain a fluorochrome with an emission maximum at 455 nm. Host caudate structures have a blue fluorescence; but green and red fluorescent structures are also seen. Counterstaining with thionin demonstrated that the blue fluorescence fibers contact nerve cells and that certain nerve cells have a blue fluorescence. Blue fluorescent structures were seen in fresh caudate slices perfused with Krebs-Ringer and following several methods of fixation of frozen caudate sections such as air drying, treatment with dimethylsulfide or dimethylsulfoxide plus diethylthiocarbamyl. The latter treatment suggests that the fluorescence does not arise from oxidation within the tissue section. Similar endogenous fluorescent structures were seen in caudate sections from mouse, rat or beef. These observations suggest that the fluorochromes have some importance in brain function and that the U.V. laser microscope may be a new approach to studying certain aspects of the microchemistry of the nervous system. 


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SOmAtic Vs. dendritic synapses: membrane resistivity is often unimportant in determining relative strengths. William R. Calvin and Katherine Grubbard, Univ. of Washington, Seattle, WA 98195.

Not all synapses are equal; indeed, length constants have been interpreted to suggest that synapses on distal dendrites are “virtually ineffective” compared to those on proximal dendrites. We have now been able to follow the way in which the axon branches and terminates within its receptive field. Neurons that respond to light touch branch repeatedly in deeper layers of the skin. The terminal branches of the axon then turn towards the surface between the skin epidermal cells. If the skin surface is viewed with Nomarski optics the touch cell endings can be seen as well-defined structures 2 - 3 µm in diameter lying between the epidermal cell profiles. An individual touch cell makes over a 1000 of these endings at the skin surface. In contrast to the touch cells neurons that respond to pressure branch less profusely within their receptive fields, and branches of the axon end in deeper layers of the skin. The use of peroxidase makes it possible to study the terminals of identified neurons by electronmicroscopy. Such information about fine structure provides a basis for further experiments on the specificity of skin innervation and reinnervation by identified mechanosensory neurons.

The goldfish M-cell (M-axon) is characterized by its large soma and a correspondingly large myelinated axon (M-axon), with the fiber diameter being 40-60 µm. These two regions are electrically coupled by a tapering axon hillock and an unmyelinated axon no more than 10-12 µm in diameter and 75 µm long. The functional role of such a constriction, which is typical of many vertebrate neurons, is not well established, although it is often assumed to electronically separate the soma and axon. We report here that the electrical properties of these different regions and their associated extracellular resistances provide for a "rectification". Specifically, there is minimal potential decrement from the soma or axon hillock to the M-axon, while there is significant attenuation in the opposite direction. This results in a low safety factor for antidromic invasion of the axon hillock but not for orthodromic activation of the M-axon.

Simultaneous intracellular recordings and current injections were used to measure input resistances of the M-cell soma and axon hillock. The input resistance of the soma is approximately 140 kΩ, while the lumped resistance of the axon hillock and somatic membranes is about 200 kΩ. In contrast, the input resistance of the caudal M-axon is at least one order of magnitude higher. Furthermore, there is minimal decrement in the amplitude of eighth nerve-evoked EPSPs from the soma to the axon (typically < 20%), while the spike recorded somatically is reduced in amplitude by 80-90%. A simple electrical model based on our resistance measurements and the geometrical relations described above shows such a "rectification". Furthermore, it provides a basis for analyzing previous observations: 1) There is a low safety factor and consequent delay for antidromic invasion of the axon hillock; 2) Antidromic invasion may spontaneously fail; and 3) Antidromic propagation into and along the M-axon is nevertheless secured with no observable delay between activation of the stimulation site and the axon hillock and the M-axon. Thus, antidromic stimulation of the M-cell may be used as a sensitive test for studying pharmacological and environmental alterations in membrane properties and may serve as a model for other regions of low safety factor, such as axonal branch points.

As part of a continuing study of the morphology and function of neurons in animals of different chromosome number, we studied the relations between internodal length (L), axon diameter (d), and total fiber diameter (D) in individual sciatic nerve fibers from triploid (2n + 1) and diploid (2n) Xenopus. Triploids (3 sets of chromosomes) were prepared by subjecting fertilized eggs to a hydrostatic pressure shock. Previous work in this laboratory and by others has shown that the nervous system and other organs of triploid amphibians contain larger, but fewer, cells than diploids of the same species. We confirmed this relation in Xenopus sciatic nerve, through fiber counts and measurements of fiber cross-sectional area.

Lengths of single fibers containing from 6 to 17 nodes were isolated after treating sciatic nerves with glycerol, and myelin dimensions were measured in 1351 internodal segments from these fibers. The relation between axon and fiber diameter was linear, but the relation was different for diploids and triploids: the average value of the ratio g = D/d was 0.625 ± 0.028 in diploids, compared to a value of 0.63 ± 0.006 in triploids, a highly significant difference. Thus the myelin sheath (D-d) was thinner around triploid fibers than in diploid fibers of the same diameter. The internodal length L was also strongly correlated with axon diameter, and again the relation was a function of ploidy: L in triploid fibers was nearly identical to L in diploid fibers of the same diameter. The volume of myelin in each internode was calculated from the values of L, d, and D, and in this case the triploids were 1.4 times those of the diploids: complex triploid had a smaller myelin volume than in diploids fibers less than 10 µm in diameter, but triploids had more myelin per internode in fibers above this size. Thus myelin morphology in animals of different ploidy is not simply scaled according to cell size differences in these classes of animals.
AN INTERACTIVE CAMERA LUCIDA COMPUTER-MICROSCOPE. E.M. Glaser, M. Glaser* and H. Van der Loos*. Institute of Anatomy, University of Lausanne, CH011 Lausanne, Switzerland.

Quantitative light microscopy has experienced a significant evolution in recent years due to its wedding with the laboratory minicomputer, a wedding facilitated by such ancillary devices as motor driven stages, video scanners, graphics displays, and graphics tablets. The resulting computer-microscopes have enabled the microscopist to acquire data rapidly and accurately and to analyze these data in a multiplicity of ways. Nonetheless, the microscopist has had to pay a substantial price for these assets: he has been divorced to a certain extent from intimate contact with the original image of the preparation. We have designed a computer-microscope system which reunites the investigator and preparation and which provides a highly flexible means for him to interact with the data acquisition and analysis procedures. The system is based upon image superimposition as provided by a camera lucida. The microscopist sees at one and the same time in a single visual field (a) the original image of the preparation, (b) a graphics display of the data acquired from this image, (c) a command 'menu' permitting him to control the system without taking his eyes from the oculars. The superimposition of the original image and the graphics display is maintained regardless of how the stepping motor driven stage is moved during the course of data acquisition. In practice, data are acquired either by stepping the stage, by 'drawing' with a graphics tablet stylus, or both. The combination of the two techniques greatly enhances the speed and accuracy of tissue examination. Because it is the original high resolution, fully chromatic image that the microscopist examines, the only optical limitations to the system are those inherent in light microscopy itself.

The computer-microscope is particularly well suited to the study of neurons stained in their entirety, as by Golgi methods. Since 3-dimensional stepping motor stage control is provided, sections of any thickness can be dealt with. The maintenance of image superimposition regardless of stage position permits viewing an unlimited expanse of the preparation with high magnification optics. This maximizes the amount of information that can be acquired. Chromatic aspects of the Image can also be studied. Other important tasks can be performed with the instrument. For example, serial sections can be aligned by letting the graphics display present the relevant contours from a neighboring section while the section on the stage is maneuvered to obtain the best registration. Another type of operation facilitates stereological analysis by having the graphics display present stereological grids selected from files stored in the computer.

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CABLE PROPERTIES, NEURONAL GEOMETRY, AND TRANSIENT POTENTIALS IN DENDRITIC SYSTEMS. Barry Honvitz, Physics Department, Texas Woman's University, Denton, Texas 76204.

A theoretical model has been developed which explores the way in which the geometrical structure of a neuron's dendritic tree affects the time course and amplitude of transient potentials generated at different locations on dendritic branches. The model is based on the work of Butz and Cowan, which allows one to investigate dendritic systems which are not of the equivalent cylinder class. We have examined the number of such systems and have developed an analytical way to evaluate the strength of the time-varying potentials that would be observed at the neuron's soma. Examples will be presented which will demonstrate how, for an input on a given branch, the potential observed at the soma depends upon such geometrical parameters as (1) the number of sister branches, (2) the branch lengths, (3) whether or not the dendrite bifurcates at a point distal to the input, and (4) given such a bifurcation, the lengths of the daughter branches.

(Supported by TIN Institutional Grant 9977)


A COMPARISON OF RAPID GOLGI STAIN QUALITY IN FRESHLY PERFUSED HIPPOCAMPAL SLICES AND HIPPOCAMPAL SLICES MAINTAINED AND STIMULATED IN VITRO. Kristen M. Harris & Timothy J. Teyler, Program in Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

A rapid Golgi staining technique for brain tissue less than 0.5 mm thick (Harris, K. M. Soc. Neurosci. Abst. 1978, 4, AI053) was applied to hippocampal slices (0.3-0.4 mm thick). One group of slices (Group A) was immediately perfused with 4% buffered paraformaldehyde and processed by this rapid Golgi technique. Other slices were placed in an incubation chamber (Allen & Teyler, Brain Res 1976, 110, 463-480) for up to 6 hours. Hippocampal slices from this second group were either stimulated (Group B) or allowed to remain undisturbed in the incubation chamber (Group C). All slices from Groups B and C were tested for spontaneous activity at the end of an incubation period. Those slices showing spontaneous activity were transferred to 4% buffered paraformaldehyde and rapid Golgi stained. Slices showing no spontaneous activity were discarded.

Qualitative light microscopic analysis of the rapid Golgi stained slices showed fully impregnated cell bodies, dendrites, dendritic spines, axons, and axonal varicosities in groups A, B, and C. Good stain quality was obtained whether the tissue was fixed in paraformaldehyde for 24 hours or for several days, from slices in all three groups.

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Qualitative light microscopic analysis of the rapid Golgi stained slices showed fully impregnated cell bodies, dendrites, dendritic spines, axons, and axonal varicosities in groups A, B, and C. Good stain quality was obtained whether the tissue was fixed in paraformaldehyde for 24 hours or for several days, from slices in all three groups.

It is suggested that this technique can be successfully applied to hippocampal slices that are experimentally manipulated in vitro. (NSF Grant BNS-78-23947)


The morphology of displaced ganglion cells in rat retina was examined as a result of filling the nerve with horseradish peroxidase by optic nerve injection. The somata of displaced ganglion cells measure between 10 and 18 μ in diameter (ave = 14.6). One or two or more primary dendritic branches arise from the vitreal surface of the soma. The primary dendrites most often branch within 20 μ of the soma, and the axon generally arises from one of the primary dendrites. Most secondary and all higher order dendritic branches are extremely fine, and except for occasional bead-like swellings, their surface is smooth and spine free. The ellipsoidal dendritic trees of these neurons as seen in flat mounted retinae averaged 240 μ in the long axis. Three examples of displaced ganglion cells drawn from three retinae are shown in the accompanying figure (arrows = axon).

In retinae embedded in celloidin and sectioned serially to the optic fiber layer, the dendrites of displaced ganglion cells are seen to ramify in the outer 1/3 to 1/2 of the inner plexiform layer. As described previously (Bunt et al., Brain Res., 91 (1975), 235), displaced ganglion cells were most common in the central portion of the retina.

Our results demonstrate a remarkable morphological homogeneity for the neurons. Staining of the central connections of displaced ganglion cells in rat and elucidation of their detailed morphology of other species should be useful in unraveling the significance of these neurons in the organization of the vertebrate visual system. (Supported by USPHS Grant EY 05185 from the National Eye Institute, and Neurobiology Ctr., CWRU, Cleveland, OH, & Marine Biological Laboratory, Woods Hole, Mass.)


Our goal is to study the forms which cytoskeletal proteins take under physiological conditions. We have chosen to study squid giant axon axoplasm because it consists principally of cytoskeletal proteins (tubulin, neurofilament proteins (NFP), and actin) and can be readily separated from its axolemma by extrusion without detergents. Additionally, the solution conditions of squid axoplasm have been defined with respect to the duality of molecular weight components. Thus, it has been possible for us to design a physiologic buffer (buffer P) which effectively replicates the solution conditions in the axon. A cylinder of fresh axoplasm was extruded from the giant axon directly into buffer P. Using SDS-PAGE, proteins diffusing into buffer P were compared to those remaining in axoplasm.

We found that axoplasmic cytoskeletal proteins have different solubilities. While essentially all of the NFP (>95%) remained in the axoplasm, most of the tubulin and actin diffused into buffer P (<5/6 and <3/4 of the total respectively). The axoplasm swelled but maintained its cylindrical morphology in buffer P for 24 hrs., at 20°C. The mitochondria were also retained in the axoplasm. Electron microscopy showed the presence of intact neurofilaments (NF) and mitochondria and the absence of microtubules. The axoplasmic structure which retains the insoluble axoplasmic proteins is subsequently referred to as the axoplasmic ghost.

Because all of the NFP remained in the ghost and electron microscopy showed the ghost to be principally NF, we conclude that essentially all axonal NFP are normally polymerized in NF and that little or no NFP are normally dissociated from the NF. A fraction of the tubulin and actin also remained attached to the ghost. This fraction must also exist as stable polymer. However, most of the tubulin and actin diffused into buffer P. This diffusible component must exist in the axon as monomers or as a polymer which does not differ physiologically. It is possible to distinguish between monomeric and polymeric forms by analyzing the kinetics of their diffusion from the ghost into the buffer. Such an analysis of the diffusion of tubulin and actin is shown when compared to the kinetics predicted by the physical equations describing diffusion for monomeric or polymeric proteins. The insoluble proteins of the axoplasm (NF) exist in the axon: diffusible monomer, soluble polymer, and stable polymer. NFP differ from both tubulin and actin in that NFP exist solely as stable polymer while tubulin and actin may exist in all three forms in the axon.


In a classic study, Furshpan and Potter (1959) showed that synaptic transmission at segmental giant motor synapses (GMS) in the crayfish CNS is electrical and that these synapses between lateral giant axon (L.G.) and axons of giant flexor motoneurons (F1) is much lower for applied orthodromic (L.G. to F1) than antidromic currents. Our recent work involving cobalt backfilling and injection of fluorescent dyes reveals new consistent differences in the gross morphology of F1 at thoracic versus abdominal synapses. At the level of the last thoracic ganglion (T8), the axis of F1 is closely apical to the L.G. for over 500 μ. At the second abdominal ganglion (A2) this "axon length" extends only to 200 μ. The cell-fusing technique in both cases is characterized by the presence of small dendrites which protrude from F1 and presumably the presence of electrical coupling. Our results are also consistent with the axon length to assess whether size in any way determines the functional characteristics of these morphologically distinct yet homologous pathways rectifying synapses. Using a microelectrode technique adapted from Ullman and Grundfest (1961) we have obtained a nearly identical decreased impedance to orthodromic currents (Rs = 1.43 ± 0.53 x 10^6 Ω) than does the A2 GMS (Rs = 6.72 ± 3.13 x 10^6 Ω). Synapses at both T8 and A2 show a nearly identical decreased impedance to orthodromic currents beyond a positive value of synaptic potential (Vs); the change to logarithmic impedance (V) is Rs ~ 10^6 Ω, however, always occurs at a lower value of Vs for thoracic (Vt = 19.9 ± 7.6 μV) than abdominal (Vs = 40.0 ± 8.3 μV) synapses. Both these features reflect an improved safety factor for transmission at thoracic giant motor synapses. Supported by NIH AM 18750.


The computer microscope was used to obtain quantitative information concerning the branching characteristics of human cortical neurons. Hippocampal pyramids from two normal human brains were analyzed, and axons and dendrites were identified by identification of spatial distribution of terminals. The infants who succumbed had similar conceptual ages (33 weeks c.a.) but differed in their maturational status of neurons. In regards to these two pre-term infants, the data suggest that (1) the average dendritic branch and fission angles of neurons from the older g.a. specimen was significantly greater than those of neurons from the younger g.a. specimen, (2) the trans-synaptic length constant of Vs when measured by applying current to L.G. and recording the membrane potential in F1 at different interelectrode distances is 3.3 ± 0.5 mm, and (3) this data strongly supports the assumption that F1 acts as an effective current source or sink along the entire contact length of the RMS. The individual length constants for L.G. and F1 were found to be greater in the older g.a. specimen; and (3) the average axonal fission angle was slightly lower in the older g.a. specimen. Information regarding the length of neuronal processes will also be presented.
CABLE PROPERTIES OF MOUSE HIPPOCAMPAL NEURONS IN CELL CULTURE.

John H. Peacock and Charles R. Walker*. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

Flow of electric current from a cell body into its branches is governed by a combination of geometric and electric factors. Estimations of distribution of current flow between soma and processes can be made from the cable equations derived by Rall (1) who has suggested that nerve cell cultures provide an opportunity to compare cable properties which have been separately determined from geometric and electrophysiologic measurements. We have studied hippocampal neurons during their first 21 days in culture. Young neurons have less extensive branching than in older cultures and are well visualized because the background nonneuronal cell layer is thin. These neurons have been studied in growth medium plus 5-10 mM added CaCl₂. The mean resting potential was -55 ± 1 mV, 98 cells; action potentials were recorded from each cell; and postsynaptic potentials occurred over the entire time span of study. Detailed measurements of cell geometry were made from photographs (1250X) with a 10X ocular. Geometric measurements were used to calculate electronic lengths (l₀) of processes and summed conductances (gₑ) of processes in N neurons under scaled end conditions for a membrane resistivity (Rₘ) of 2000 Ωcm² and an internal resistivity of 70 Ωcm. Electrophysiologic data give input resistance (Rᵢ), membrane time constants (τᵢ), and estimates of electronic length for the entire neuron (lₑ).

In 3 cells, measured Rᵢ was compared to predicted values, from branching analysis. Hered, Rᵢ's of 1725, 1610, and 610 Ωcm² plus detailed geometry which the Rᵢ's of 100, 100, and 106 Ns respectively. Furthermore, predictions of Rᵢ from electrophysiology (see Rall, 1) are respectively 1330, 1620, and 640 Ωcm²; hence, geometric and electrophysiologic measurements of Rᵢ and Rᵢₑ should reasonably well in these cells. The issue of whether cable properties change with development in culture will be addressed in subsequent work. (Supported by NIH grants NS 12151 and NS 107012.)


SYNAPTIC ULTRASTRUCTURE OF SINGLE PHYSIOLOGICALLY IDENTIFIED NEURONS USING COBALT. Christine E. Phillips, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England.

Techniques for intracellular recording and iontophoresis of cobalt dye into identified neurons in the locust Schistocerca americana in in vitro cultures have been in routine use for the past 8 years. Many motoneurones to flight and leg muscles and some of the pre- synaptic interneurones have been physiologically characterised and their morphology examined at the level of the light microscope after silver intensified cobalt staining. A technique has been developed to preserve the synaptic structures of dye marked motoneurones and local non-spiking interneurones. Electrodes containing a mixture of cobalt chloride and potassium chloride were used for recording, physiological identification and staining of the neurons. Ganglia were prefixed prior to subfide precipitation of the cobalt, then fixed for electron microscopy and embedded in Epon 812. Ganglia were serially sectioned at 2.5um. Sections were silver intensified on glass slides, photographed and re-embedded. Thin sections were stained with uranyl acetate and lead citrate and photographed in a Philips EM300.

The cobalt-silver complex appears as discrete dense particles ranging from 15nm to 40nm in diameter. The highest concentration was found at the periphery of most motoneurones and local non-spiking interneurones. The densest areas were more consistent with the cell bodies of neurons. Ganglia were post fixed and stained, with average Rᵢ, 27.8 ± 7.4 (mean ± S.D., megohm) for the entire neuron and could be found in terminal arborisations. Using this technique, synaptic structures of identified motoneurones and presynaptic, non-spiking interneurones in the mesothoracic and metathoracic ganglia of the locust have been clearly seen. This work was supported by S.I.H. postdoctoral fellowship No. 1 F 32 NS 06028 01.


Input resistance (Rᵢ) and other intracellular parameters were measured in CA1 cells in the in vitro hippocampal slice preparation. Subsequently, horseradish peroxidase was injected intracellularly and then reacted with diaminobenzidine and H₂O₂. Twelve pyramidal cells and 2 interneurons were successfully stained, with average Rᵢ, 27.8 ± 7.4 (mean ± S.D., megohm) for the pyramidal cells and 27.4 ± 10.8 for the interneurons. The specific quantitative anatomy of each cell was then reconstructed using computerized finite cable analysis (Rall, W. Exp. Neurol. 1:491, 1959). Specific membrane resistance (Rm, ohm-cm²) averaged 2450 and 4860 for the pyramidal cells, comparing sealed-end and infinite infinite cable terminations, respectively. Rᵢ ranged between 915 (sealed-end) and 1050 (infinite cable termination) for the 2 interneurons, one of which appeared to be a basket cell histologically. Evaluation of the three-multipole of the diameters at branch points revealed an average of 1.001, for 2680 branch points.

Aphal dendrites of pyramidal cells terminated at 1.2-2.5 length constants, while the basilar dendrites ended at 0.7-2.0. For voltage injected at the most distal apical and basilar dendritic terminations, only 0.4% would appear at the soma. For terminals within the stratum radiatum, 3.3% of the injected voltage would reach the soma. However, for current injected at the apical terminations, an average of 3.4% would arrive at the soma, as compared to 51.2% for the most distal basilar dendritic regions and 72% for the stratum radiatum terminations. Input resistances of these apical and basilar dendritic segments averaged 2.9kΩ ± 0.8 ohms. Thus, current in these cells achieves a higher transfer efficiency than voltage in electrophoretic conduction from distal parts of the cell to the soma. The dendritic to soma conductance ratio in pyramidal cells averaged 3.5 ± 0.3 for the sealed end terminations and 7.7 ± 0.1 for the infinite cable termination. These measurements compare well with those on the motoneuron, and indicate that distal synaptic events may significantly influence ongoing activity at the soma, without active conduction.
NEUROPATHOLOGY AND NEUROIMMUNOLOGY
IMPAIRED PROLIFERATIVE RESPONSE OF LYMPHOCYTES TO MEASLES ANTIGENS IN MULTIPLE SCLEROSIS.


Two factors appeared to be most important for successful cell proliferation to measles antigens: 1) the optimal cell concentration (1 x 10^6 cells/ml) and 2) the selection of a correct batch of human AB+ plasma. The HI titers to measles antigens in patients with multiple sclerosis (MS) and compared to that in 23 normal controls and in patients with other neurological diseases. The blastogenic index to measles antigens was studied in 23 patients with multiple sclerosis (MS) and compared to that in 23 normal controls and in patients with other neurological diseases. MS patients exhibited a consistently low response to measles antigens compared to normal controls. However, responses to mumps, parainfluenza, and spinal cord were normal in appearance. Supported by NIH Grant NS 13042.


The blastogenic response of lymphocytes to measles antigens was studied in 23 patients with multiple sclerosis (MS) and compared to that in 23 normal controls and in patients with other neurological diseases. MS patients exhibited a consistently low response to measles antigens compared to normal controls. However, responses to mumps, parainfluenza, and spinal cord were normal in appearance. Supported by NIH Grant NS 13042.


Two factors appeared to be most important for successful cell proliferation to measles antigens: 1) the optimal cell concentration (1 x 10^6 cells/ml) and 2) the selection of a correct batch of human AB+ plasma. The HI titers to measles antigens were compared when controls to normals (mean response 45% lower than controls). However, responses to mumps, parainfluenza, and spinal cord were normal in appearance. Supported by NIH Grant NS 13042.


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MULTIPLE FORMS OF THE COPPER(II)-CARNOSINE COMPLEX AND THEIR POSSIBLE INVOLVEMENT IN WILSON'S DISEASE.


The copper(II)-carnosine complex has been well characterized in the crystal and as an electronically coupled dimer that is chelated by two molecules of carnosine (β-alanyl-L-histidine). The complex in solution was assumed to consist only of a single copper(II) ion chelated to one molecule of carnosine with crystallization occurring when two of these complexes joined to form the dimer. Since the monomeric complex in solution was shown to be very unstable, no such complex could be found in vivo. We have found that the copper monomer is formed in solution only when carnosine is in excess. There are four molecules of carnosine bound per copper(II) ion instead of only one as indicated by the nitrogen hyperfine splitting of the 9G low field isotope transition in the S-band ESR spectrum at 77K. (Fig. 1). In addition, the

It has been shown in the rat by Blakemore (Nature 266:68,1977) that segments of the autologous sciatic nerve can be transplanted into regions of the spinal cord. The aim of the present study was to determine the feasibility of grafting cultured xenogenic Schwann cells to the mouse demyelinated spinal cord.

Laminectomies were performed on C57BL/6 mice and quaking (qk) mice in mid-lower thoracic area and the dura exposed and then incised. A hand held 20 µm tip microsyringe connected to a microsyringe containing lysophosphatidil choline (LPC) (10 µg/ml) was inserted into the dorsal columns and 1 µl injected at three sites. A small breach in the plia was made in the central area of this area. Holtzman rat Schwann cells, cultured and separated from neurites (Brain Res. 300:169) were then inserted into this breach and teased gently under the_pro. The wound was closed and the animals allowed to survive from periods of between 2 and 15 weeks during which the rats were carried out by twice weekly injection of host mice with antilymphocytic serum (ALS). From 2 weeks it was possible to detect spinal cord axons which were myelinated by Schwann cells. Myelination by Schwann cells was observed to extend along the dorsal column of the spinal cord for a distance of approximately 200 µm, rostral and caudal to the site of the graft. These were especially obvious in the qk mice where axons well myelinated by Schwann cells were adjacent to and mixed with the host's hypomyelinated axons.

Early evidence that these Schwann cells were foreign was deduced by graft rejection techniques. Our findings suggest that Schwann cells grown in vitro can ensheath and myelinate both demyelinated axons in the CNS as well as regenerating axons in the PNS (Aguayo et al, Neurology 29:589, 1979).


It has recently been reported (Brain, 101:661, 1978) that patients with Parkinson's disease lack a severe and long-lasting visual evoked potential (VER) latencies than nonparkinsonian patients. This altered response to photic stimuli may result from the brain damage following long-lasting Parkinson's disease, or from other nonselective factors. We report here that brain dopamine depletion significantly increases latency of the VER evoked potential in the visual cortex of rats. Chronically prepared rats were injected with the following drugs, presented in counterbalanced order once every three days: Alpha-methyl-para-tyrosine (AMT) (75 mg/kg 6 hrs + 75 mg/kg 2 hrs before testing), FLA-63 (25 mg/kg 4 hrs before testing), scopolamine (2 mg/kg 15 min before testing), saline (2 ml/kg 6 hrs + 2 ml/kg 15 min before testing). In a separate experiment, ten animals were injected with (150 mg/kg) para-chlorophenylalanine (PCPA) and ten others were injected with saline. In this experiment all injections were given 3 days before testing. Peak-to-peak amplitudes and peak latencies of the potential evoked by 64 flashes were recorded. AMT significantly increased latency to the first negative peak (N1) (34.5±0.8 msec) compared to the saline control (30.1±0.3 msec), while FLA-63 did not alter latency (30.6±0.4 msec). AMT also increased latency of peaks P1 and P2. Dopamine depletion was apparently responsible for the altered latency, since depletion of norepinephrine by FLA-63, a dopamine-β-hydroxylase inhibitor, did not produce any change. AMT and FLA-63 findings suggest that schizophrenia effects of later peaks (N2, P3 or N3), thus demonstrating independence of these peaks from earlier ones (P1, N1, P2). Since the effect of AMT occurs in the more evoked potential, it is possible that the retina contains dopamine, and since thioridazine has been shown to increase the latency of the VER A wave in humans (Int. Pharmacopsychiatry, 1:3, 1978), it seems likely that the effect of AMT on the visual evoked potential occurs primarily at the retinal level. This interpretation is supported by the finding that the amplitudes and latencies of prolonged potentials recorded in the cortex following optic tract stimulation.

1715 Long-Term leanInG deFicit in the raT PrOduCed afTeR MethylMercury exposure in late GestaTion. C.U. Eccles and Z. Annau. The Johns Hopkins University, School of Hygiene and Public Health Baltimore, MD 21205.

Prenatal exposure to methyl mercury (MeHg) is known to cause central nervous system damage in a variety of species. The nature of the resulting deficits and the parameters involved in the manifestation of them is currently under investigation. A previous report from our laboratory demonstrated that the offspring of rats treated with 276 g MeHg on day 7 of gestation did not meet the criterion required significantly more criterion level of responding during the acquisition period. The greater variability in the MeHg treated group for this measure.

3 days after administration of MeHg on gestational day 14 were more severe than those observed after administration on day 7.

This is a report on a method for producing rats which spontaneously and voluntarily ingest ethanol. The morphology of the brains and livers from a population of these animals has been examined. In addition, their liver alcohol dehydrogenase (ADH) activity has been measured. These studies are currently under investigation.

Thirty-two rats were housed in a semi-naturalistic rat colony environment with ad lib access to 10% ethanol and water. After seven months of colony housing, all animals were captured, placed in individual cages and alcohol and water consumption were monitored. There was a wide range of alcohol appetites; five animals showed a marked alcohol preference (4.5-8.1 g/day) while another eight animals consumed virtually no alcohol (0.5-0.7 g/day). The remaining nineteen animals consumed intermediate amounts of alcohol (0.9-4.5 g/day). These extremes of alcohol preference were apparently due to the colony housing since they did not occur in twenty-seven rats kept in isolation for the same length of time and also offered ad lib access to ethanol and water. Blood ethanol levels, spontaneous withdrawal signs, food consumption, and behavioral studies are currently being examined in a similar colony.

After the seven months in the colony, the animals were sacrificed and the brains and the livers were removed. The morphology of the nervous system was examined on an ultrastructural level and correlated with a Golgi study and a conventional light microscopic study. Structural alterations have been observed in the hippocampus, the mammillary bodies, the cerebral cortex, and the cerebellar cortex of the alcohol consuming rats. These alterations include numerous dendritic varicosities which contain smooth membranous vesicles. Some of these vesicles are continuous with the dendritic membrane while others are contiguous but not connected to the dendrite. In the liver, the morphology of the livers from the alcohol consuming rats has also revealed structural alterations. Light microscopic examinations have indicated normal hepatic architecture in the animals that did not consume alcohol and a fatty metamorphosis in the alcohol consuming rats. The fine structure of these livers is currently being examined. In addition to the morphological studies of the liver, liver alcohol dehydrogenase activity was examined. The alcohol consuming rats had significantly higher liver ADH levels. (Supported by Grant AA04513 from the National Institute on Alcohol Abuse and Alcoholism.)


Recent evidence indicates that the pathogenesis of myasthenia gravis (MG) involves antibodies directed against nicotinic acetylcholine receptors (AChR). Antibodies to the AChR in both MG and an animal model of the disease, designated experimental autoimmune myasthenia gravis (EAMG), are routinely measured using a radioimmunoassay (RIA) utilizing [125I]-labelled α-bungarotoxin (a-BGT) as a specific label for the AChR. We have developed an alternative method for the quantification of both AChR and anti-AChR immunoglobulins employing the enzyme-linked immunosorbent assay (ELISA).

In its simplest form, purified AChR is adsorbed to the wells of a polystyrene microtitre plate by incubation in carbonate buffer. This is followed by sequential incubation with excess anti-AChR serum, peroxidase-conjugated anti-immunoglobulins, and a suitable substrate. The degree of substrate catalysis is then determined by optical density measurements. This procedure can be adapted to measure AChR or anti-AChR immunoglobulins.

If an additional reagent, the use is also possible to quantitate specific classes of anti-AChR immunoglobulins. If a-BGT is adsorbed to the polystyrene, followed by AChR in phosphate buffer, and sequential incubations with reagents as described above, the assay becomes more broadly applicable.

CONTENT AND FUNCTIONAL CAPACITY OF CEREBELLAR RNA FROM RATS WITH GRAFT VERSUS HOST DISEASE (GVHD). W. J. P. Griffin, M. Morrison*, and J. R. Head*. University of Texas Health Science Center at Dallas, Texas 75235.

The possibility of adverse effects on developing fetuses due to the passage of mature lymphocytes from the maternal to fetal circulation has long been recognized. Such passage can result in GVHD occurring during the period of brain development. However, our series of experiments, utilizing the rat cerebellum as a model, is the first to investigate the effects of such an immunological assault on the developing nervous system. We simulate maternal-fetal cell passage by intravenous injections of 40 x 10^6 parental strain lymph node cells (PBLNC) from Fischer rats (F1) into (F1 x DAF) hybrid rat pups on the day of birth. The F1 hybrids do not recognize the PBLNC as nonsensel, but the PBLNC recognize the DA antigen present on the F1 cells and attack host lymphoid tissue, the first of a cascade of events called GVHD.

We have previously reported that GVHD decreased cerebellar RNA synthesis, but did not determine the various RNA species and the ability of RNA to direct protein synthesis in a cell-free wheat germ culture). During acute GVHD (postnatal day 14 there was a decrease in 1) the amount of RNA as shown by less staining with cresyl violet of cerebellar sections from animals with GVHD when compared to litter mate controls, 2) total RNA content (65% of control) as measured by a spectrophotometric method, 3) 3H-uridine uptake into the RNA fraction (65% of control), and 4) the overall translational capacity of RNA (85% the biological activity of the control). We have also revealed structural alterations. Light microscopic examination of the liver, liver alcohol dehydrogenase activity was examined. The alcohol consuming rats had significantly higher liver ADH levels. (Supported by Grant #AA03513 from the National Institute on Alcohol Abuse and Alcoholism.)

EFFECTS OF LOcus COeruleus AND ANTERIOR hypothalamic BRAIN LESIONS ON ANTIBODY FORMATION IN MICE. Nicholas R. Hall, John R. Lewis*, Richard T. Smith* and Steven F. Zornetzer. Dept. Pathol. University of Florida College of Medicine, Gainesville, Florida 32607.

Previous data have shown that electrophoretic lesions of the nucleus locus coeruleus are capable of inhibiting the subsequent formation of both gamma globulin and gamma globulin antibody specificities. These effects on antibody formation have been postulated to be mediated by the regulation of the immune response. Here, we report the effects of lesions of the locus coeruleus and the anterior hypothalamus on antibody formation. A number of experimental parameters were investigated which might have been due to cyclic fluctuations of ovarian hormones. Animals were killed at the end of the experiment.

The experimental animals were 250 grams of male C57B/6J mice with bilateral lesions of the nucleus locus coeruleus or the anterior hypothalamus. The animals were killed at the end of the experiment.

Animals in both lesion groups were found to be good antibody responders. There was no statistical difference between those animals that had received lesions of the locus coeruleus and those with anterior hypothalamic lesions. Neither of the values obtained from the lesioned rats differed from the unlesioned control. These data suggest that the inhibitory influence of locus coeruleus lesions upon bone marrow stem cells is not manifested by a functional lesion of the immune system as assessed by using the above paradigm.
THE RAPID AVOIDANCE TEST OF GERBILS AFTER UNILATERAL CEREBRAL ISCHEMIA. Charles J. Hannan Jr., Andrew J. Lloyd and John J. McCloskey. Clinical Investigation and Research Division, and Pathology Department, Eisenhower Army Medical Center, Ft. Gordon, GA 30905.

Ninety male Mongolian gerbils completed 6 daily evaluations on the rapid avoidance test (RAT) in an attempt to quantify the effects of cerebral ischemia produced by occluding a common carotid artery. Animals were trained in a shuttle box on a RAT schedule of 4 escape trials (tone and shock presented simultaneously for 40 sec) followed by 2 avoidance trials (20 sec of tone then 20 sec of tone and shock) with a 5 minute intertrial interval. Criteria for entry into the study was at least one successful response in the avoidance trials. One hundred and ninety six gerbils were trained with 26.5% rejected for not meeting the criterion. Animals were randomly assigned to groups for 1) right, or 2) left carotid artery occlusion, ad 3) surgical controls. Animals were given the RAT just prior to surgery (animals failing to avoid were also rejected); at 3.5, or 7 hours after surgery; and then on 1, 2, 3, and 6 days post surgery. If animals died or were judged to be physically unable to perform the RAT they were eliminated from the study and additional animals were trained so that each group contained at least 30 animals at the end of the study. Of 104 animals entered into the occlusion groups, 40 died (right occlusion 17/49, left 23/55), therefore 64 occluded animals are histologically demonstrated in 14 of the 64 (21.9%) surviving animals (9 left, 5 right hemisphere). Mean values of performance on the RAT exhibited differences between groups, with animals which were found to be infarcted doing poorer than non infarcted or control animals, and left side infarcted animals doing worse than those with right infarcts. No measure of escape/avoidance time or patterns of time was uniquely predictive of infarction, although significant population tendencies were found.

1722 IMMUNOCYTOCHEMICAL OBSERVATIONS ON DEMYELINATING LESIONS IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). Yasuto Itoyama*, Henry deC. Webster. NINDS, NIH, Bethesda, MD 20205.

Focal changes in the distribution of myelin sheath constituents that occur during myelin breakdown are poorly understood. To investigate these changes, we induced experimental allergic encephalomyelitis (EAE) by injecting an emulsion of spinal cord and complete Freund's adjuvant into Lewis rats. In guinea pigs, the first symptoms occurred 10-11 days after injection; all of them had a rapidly progressive illness and died on the 15-16th day. The rats became ill after 10-15 days; their illness was more severe 3-4 days later and all of them were recovering when sacrificed 17-19 days after injection. Animals were perfused with fixative before symptoms appeared, within 48 hrs of the first symptoms, and during severe illness. Rats also were perfused during recovery. Vibratome and paraffin sections were immunostained with antisera to myelin basic protein (BP), a major constituent of compact myelin, myelin-associated glycoprotein (MAG), located perinervally in myelinated fibers, and immunoglobulin G (IgG) according to our modification of the peroxidase-antiperoxidase (PAP) method. The major findings were: 1) Early focal fragmentation of myelin sheaths was easier to identify with BP immunostaining than with conventional histochemical methods. 2) In more advanced lesions, fragmenting sheaths and demyelination were found beyond margins of perivascular infiltrations especially in the gray matter. b) Demyelinating lesions in the gray matter also contained substantially fewer mononuclear cells than the white matter lesions of the same size. 4) In periventricular lesions, myelination was shown to progress extracellularly and in subependymal macrophages. Some macrophages were found between ependymal cells and a few were located intraventricularly. 5) In zones of myelin fragmentation and breakdown, perivascular staining for macrophage marker was present also. In early EAE lesions, IgG was present perivascularly. Its penetration into the surrounding parenchyma increased as the lesions progressed. The IgG staining pattern was similar to that reported by others for EAE blood brain barrier experiments using tracers.

1723 TREMETHADIONE INCREASES THE REDUCTION IN BRAIN MICROVASCULAR VOLUME AFTER PROLONGED CEREBRAL ISCHEMIA IN GERBILS. D. Jarrett*, P. B. Dumas. Dept. of Neurosurgery & Pharmacology, Tulane Univ. School of Medicine, New Orleans, La. 70112.

Qualitative methods of outlining the cerebral microcirculation with intravascular India ink have been used to describe a 'no-reflow phenomenon' following ischemia. In this process reperfusion of the ATP-depleted brain is hampered by microvascular occlusion. Animals were perfused immediately following 1.5 to 15 minutes of reversible complete cerebral ischemia both with and without subsequent post-ischemic reperfusion. In all animals perfused by microvascular occlusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed.


Adult cats were subjected to 1.5 to 15 minutes of reversible complete cerebral ischemia both with and without subsequent post-ischemic reperfusion. In all animals perfused by microvascular occlusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed. In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both with and without subsequent post-ischemic reperfusion no reperfusion was observed.

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CATALASE ACTIVITY IN NORMAL AND DERENERVATED HEREDITARY DYSTROPHIC HAMSTER MUSCLE. Robert R. Jenkins and Diane Newsham*. Biology Dept., Iowa State University, Ames, IA 50011.

Catalase (H$_2$O$_2$ oxidoreductase, E.C. 1.1.1.6) has been shown to be present in normal muscle (Staurer and Schottelius, Exp. Neurol. 48: 524-33, 1975). The exact biological role of catalase has eluded investigators despite the voluminous amount of research which has been conducted on this enzyme. Work from the lab of Staurer et al. (Exp. Neurol. 59: 381-89, 1977) and our own lab has shown that catalase activity increases during various forms of muscle wasting such as denervation, cancer and denervation. The studies also suggest that this type of hypercontraction develops in vivo, but it does not necessarily lead to necrosis.
1729 LOCAL INJECTION OF lysophosphatidyl choline (LPC) INTO THE CORPUS CALLOSUM OF the rabbit produces a focal demyelinating lesion. LPC (1% in normal saline) was injected in volumes of 1-4 µl through a glass micropipette (tip diam. 10 to 30 µ) fixed to a microsyringe. The LPC was delivered over a period of 10-40 min. Survival times ranged from 1 hr. to 6 weeks. The lesions were assessed histologically using the Luxol fast blue stain for myelin and the Holmes silver nitrate method for axon cylinders. With these injection methods, the entire vertical extent (about 0.5 mm) of the corpus callosum could be demyelinated with minimal amounts of axonal degeneration. Ultrastructural analysis reveals pathological changes in the demyelinated zone ranging from sparse axonal degeneration to complete demyelination.

In some animals chronic stimulating electrodes were implanted in the corpus callosum near the midline and field potential recordings were obtained from the LPC injected limb. The pipette was positioned at a point corresponding to the maximal negativity of the evoked callosal field potential, and LPC was injected at this level. After the injection the callosal field potentials were recorded with two EEG electrodes on consecutive days from the same animal. The fields evoked from single stimuli were reduced in amplitude minutes after LPC injection and this reduction persisted for several days. Another effect of the LPC injection was the reduction in the amplitude of the response evoked by the second of two paired stimuli at interstimulus intervals of up to several hundred msec. This is in contrast to the evoked response in control animals where the amplitude reduction occurs only with interstimulus intervals of up to about 3.0 msec. The present study indicates that it is possible to produce focal demyelinating lesions in fine caliber myelinated axons of the mammalian cerebral cortex. Therefore, it will be possible to study, over protracted periods of time, the physiological and morphological properties of focally demyelinated cerebral axons.

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1730 BEHAVIORAL DEFICIT OF MICRENCEPHALIC RATS IN AVERSIVELY MOTIVATED LEARNING. Moon Hee Lee, R. reddad, Anusa Kabe and Ruth Duman*. Neuropharmacology Laboratory, New York State Institute for Basic Research in Mental Retardation, Staten Island, NY 10314.

Micrencephalic rats, the progeny of rats injected with 30 mg/kg of methylazoxymethanol acetate (MAM Ac) on gestation day 15, are deficient in learning to reverse a previously learned position habit in a T maze. This deficit, originally observed in adult rats, has since been demonstrated in immature rats both before and after weaning. In all of these experiments, appetitive motivation was used. However, we have recently found that micrencephalic rats that had to learn to reverse a previously learned position habit to escape from a water filled T maze differ much more sharply from the normal rat. Similar results were obtained both before and after weaning. These findings, along with the previously reported differences in "behavioral arousal" of the micrencephalic rat (Rade & Haddad, Federation Proceedings, 1972, 31: 1598-1599), suggest that, regardless of any deficit in cognitive function, micrencephalic rats (produced by prenatal treatment with MAM Ac) differ in motivational and/or emotional aspects. The behavioral differences are consistent with those that might be expected to result from the alterations in neurotrophines in the cerebral cortex of the micrencephalic rat reported by Johnston et al. (Science, 1979, 203: 369-371). It would appear that estimates of the nature and extent of cognitive and functional deficits in micrencephalic subjects may be grossly in error if the methods of appraisal do not take into account their altered emotional reactivity.


DFP is toxic to peripheral nerves and produces a localized mono-neuropathy by intravenous injection of DFP has been described (Lowndes et al. Europ. J. Pharmacol. 29: 66, 1974) and morphologically characterized (Glazer et al. J. Neurocytology. 7: 8. 1978). The present studies were performed to confirm that the neurotoxin remained largely confined to the periphery of the injected limb, thus ruling out a local effect of cell bodies in the etiology of the neuropathy. DFP (2 mg/kg body wt) labelled with 3H-DFP (5 µCi/kg) was injected into the left femoral arteries of cats. On day 1, 3, 5, 7, 10, 12 and 28 days after lisolecithin injection there is a central area of necrosis, free of viable cells, surrounded by a large area in which there is virtually complete myelin breakdown and destruction of glial cells and invasion by a few macrophages with relatively good preservation of axons. By day 3, numerous lipid laden macrophages are present throughout the lesion. Many of the axons are denuded of myelin while others are surrounded by degenerating myelin remnants. There is already a suggestion of beginning remyelination at 5 days and easily recognizable remyelination is evident by day 28. Mitotic glial cells were scarce day 1. A few were seen at day 3, but they were more numerous at day 5 and day 8, apparently decreasing in numbers subsequently. The rapid appearance of dividing oligodendrogliia which can be clearly identified by electron microscopy three days after lisolecithin injection, suggests that the cell proliferation may begin as a direct response to injury rather than requiring induction by denuded axons.

1732 CELL PROLIFERATION AND REMYELINATION. Lilia M. Mardas and Robert M. Hermann Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY 14624.

The processes of cell proliferation and remyelination were studied in 1 month old rats using mitotic arrest with colchicine to identify proliferating cells. Demyelination was induced by the focal injection into the spinal cord of 2 µl of a 10 mg/ml solution of lisolecithin containing a small quantity of colloidal carbon to serve as a marker. 200 µg of colchicine was injected intraperitoneally 20 to 22 hrs before the animals were sacrificed. This procedure produced arrest in metaphase of any cells entering mitosis during this period. Animals were killed by perfusion at 1, 3, 5, 8, 12 and 28 days after lisolecithin. 24 hrs after lisolecithin injection there is a central area of necrosis, free of viable cells, surrounded by a large area in which there is virtually complete myelin breakdown and destruction of glial cells and invasion by a few macrophages with relatively good preservation of axons. By day 3, numerous lipid laden macrophages are present throughout the lesion. Many of the axons are denuded of myelin while others are surrounded by degenerating myelin remnants. There is already a suggestion of beginning remyelination at 5 days and easily recognizable remyelination is evident by day 28. Mitotic glial cells were scarce day 1. A few were seen at day 3, but they were more numerous at day 5 and day 8, apparently decreasing in numbers subsequently. The rapid appearance of dividing oligodendrogliia which can be clearly identified by electron microscopy three days after lisolecithin injection, suggests that the cell proliferation may begin as a direct response to injury rather than requiring induction by denuded axons.

Review of the literature offers a good deal of physiological or biochemical studies regarding changes in the central nervous system, secondary to alcohol administration. On the other hand, recent anatomical studies are less common and, as far as we know, the tridimensional status shown by neurons, their prolifera-
gations and the neuropile as a whole, has not been described.

We have been studying, with Golgi methods and some other neu-
rohistological techniques, the morphological changes that have
developed in brains of alcoholic rats. For this purpose, we have
raised three groups of rats under the following conditions:
Control group: this group has been allowed to eat regular pellets
and drink, ad libitum, common tap water. Experimental group A :
this one also eats regular pellets and drink from a 10% alcohol solution. Experimental group B: these rats also receive the
same type of pellets and drink from a 20% alcohol solution, as
the only source of liquid. They have been kept, under constant
conditions, for over a year. The liquid intake has been recor-
ded every day, and the weight increment every other day.

We have been impressed by the quantity and quality of chan-
ges occurring in the CNS of these rats. In this communication we
describe the observations made in the hippocampal-dentate com-
plex. A new type of astrocyte, including microglia, is between the
Sleer and spineless dendrites. 3. Swollen and fragmented dendrites. 4. Enlarged mossy tufts (terminals), and 5. Evidence of dendritic
proliferation with abnormal features. These changes are not
age-related. The possible functional significance of these
changes shall be discussed.

This study has been supported by "Centro Mexicano de Estu-
dios en Salud Mental".

1734 DICESTHALIC INJECTIONS OF KAICID ACID PRODUCE MYOCARDIAL NECRO-
SIS. Patrick L. McGeer, C. Kel Galabru*, Edith G. McGeer and
William J. Borko*. Klinicke Laboratory Neurological Research, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5, and
St. Paul's Hospital, Vancouver, B.C., Canada.

Bilateral injections of 3 smoles of kastic acid (KA) into the
thalamus produce the following triad of peripheral consequences: 1) periarteriolar myocardial necrosis, 2) elevated serum fibrinon-
gen, 3) gross hematuria. Electrolytic lesions of the thalamus
and intracerebral injections of KA at several other locations
fail to produce this result. KA at much higher doses subcutane-
ously or intraperitoneally is also inactive. Some protection
against myocardial damage is produced by reserpine or 6-hydroxy-
dopamine, but atropine confers no protection. Urinary noradre-
saline levels are markedly elevated following effective but not
Ineffective IA. injections suggesting sympathetic "storm" may be
one factor. Elevated serum fibrinogen is presumed to be related
to tissue damage, while gross hematuria comes from bladder
necrosis. The fact that myocardial damage may result from intra-
cerebral lesions and/or pathological stimulation by KA may have
clinical implications. Cardiac damage occasionally results in
humans from strokes and intracerebral hemorrhage and no satis-
factory explanation has ever been offered for the phenomenon
of interstitial myocardial fibrosis. Such an end result might
follow on repeated stress of cerebral origin. (Supported by

1735 VIRUS-INDUCED IMMUNORETINOPATHY IN NEWBORN RATS. Andrew A.
Monjan and Manuel del Cerro, Department of Epidemiology, School of Public Health, Johns Hopkins University, Baltimore, MD and
Center for Brain Research, University of Rochester School of
Medicine, Rochester, NY 14642

A destructive developmental retinopathy can be induced by
infecting rats, within the first 2 days of life, with lymphocytic
choriomeningitis virus (LCMV). The lesion is immunologically
mediated as attested by the sparing effects of neonatal thym-
ectomy or anti lymphocyte serum. We now present a correlative
optical and electron microscopic histopathological analysis of
the changes occurring in the LCMV retinopathy. These data indi-
 cate that there are, within the cell population of the retina,
various degrees of sensitivity to the immune reaction to LCMV.
Thus, within the neural retina, there is a distinct gradient of
degeneration from the outer to the inner layers. The rods are
the least resistant with the ganglion cells being the most per-
sistent of the neurons. Muller glial cells first react with a
hypertrophic proliferation of the cytoplasmic lamellae which
fill up some of the space vacated by the destroyed neurons and
later they undergo pronounced atrophy. The least affected
elements are the retinal pigment epithelial cells which, though
infected, invariably survive, with comparative minor alterations,
even after all the neuronal cells have degenerated. It is note-
worthy that the progress of the lesion through the layers fol-
ows an exactly opposite direction than that of the spread of the viral antigen as observed by immunity fluorescence. Thus, while at
10 post-infection days, the antigen is still localized in the inner nuclear layer, morphological alterations are only seen in the
outermost regions of the outer nuclear layer.

Finally, although the fluorescent antibody technique shows
extensive distribution of the LCMV antigen, no unequivocal
identification of viral particles has been made yet. This fact
stands in sharp contrast with the profuse presence of viral
particles observed in the LCMV infection of the immature cere-
bral cortex. Work is in progress to determine whether this dif-
erence originates from a sampling problem or represents a unique feature of the LCMV retinopathy.

Supported by National Eye Institute Grant 5 KY 02632-01

1736 EARLY SEGMENTAL DEMELINATION IN EXPERIMENTAL DIABETIC NEUROPATHY
S.A. Moore*, R.S. Peterson, D.L. Feltin, and B.L. O'Connor*,
Anatomy Department, Indiana University School of Medicine,
Indianapolis, Indiana 46223

Studies in this lab revealed a small but significant number of
nerve fibers undergoing segmental demyelination (SD) in nerves
showing a reduced nerve conduction velocity (CV) in 2 month dia-
abetic rats made diabetic by alloxan and streptozotocin (STZ).
Although SD is a consistent pathological finding that may account
for reduced CV in diabetic humans, its presence in animal models
is disputed even though decreased CV in these models is well
documented.

As part of a comprehensive, long term, longitudinal study, the
sural and tibial nerves from the mid-calf region of 2 month STZ
and alloxan diabetic rats were studied using a teased fiber
preparation and compared to age matched controls. Approximately
50 nerve fibers were teased from one sural and one tibial nerve of
9 STZ, 9 alloxan, and 11 control rats. A total of 13 nerve
fibers from 8 of the diabetic rats showed evidence of SD. Only
one nerve fiber in one control rat had similar evidence. Other
pathology observed in diabetic nerves included myelin globules
indicative of Wallerian degeneration, swollen axons, and appar-
ent loop formation in internodal myelin.

Although the number of SD nerve fibers may seem small, it is
significant in that only a small percentage (about 5%) of the
total number of fibers was sampled and a relatively short length
(<10% of total) of nerve was examined. Many more nerve fibers
than those examined (as well as other areas of those fibers that
were studied) may be involved in a demyelinating process.

That a significant number of nerve fibers is involved is sup-
ported by CV studies done in this lab on the same nerves show-
ing evidence of SD. While as yet tentative, these studies re-
vealed a slowing of CV in the sural nerve but not in the tibial,
suggesting a preferential sensory involvement.

These data, suggesting preferential slowing of sensory CV that
is accompanied by a possible morphologic correlate (SD), corre-
spond well with descriptions of human diabetic neuropathy in
the literature. In light of this, it is important to determine the
fibers induced by STZ and alloxan appear to be appropriate models for
the investigation of human diabetic neuropathy.

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ENDONEURIAL FLUID PRESSURE DYNAMICS
Robert R. Myers, Henry C. Powell*, Michael L. Costello*
Veterans Administration Hospital and the University of California, San Diego, Departments of Anesthesiology, Neurosciences and Neuropathology, La Jolla, CA 92037.

In this study we advance hypotheses which may postulate record endoneurial fluid pressure (EFP) and have thus allowed investigation of nervous system transport phenomena in the peripheral nerve system, processes which is more amenable to control and experimental manipulation than the brain. We have employed the active, selective, nerve-endoneurial fluid system to record rat sciatic nerve pressures in four experimental groups of animals to explore the mechanisms of edema in peripheral neuropathy and to quantify these physical relations between EFP and control. EFP was 2.0 ± 1.0 cm H2O for adult, Sprague-Dawley rats. Significant differences were observed between controls and the following experimental groups:

**Insult**

<table>
<thead>
<tr>
<th>4% pO2:in water</th>
<th>6% saline</th>
<th>proximal crush injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000 ppm hexachlorophene in diet</td>
<td>10 cm H2O</td>
<td>9 cm H2O</td>
</tr>
</tbody>
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These studies reveal several mechanisms responsible for increased EFP. The blood-nervous barrier (BNB) may be damaged by toxic injury to the endothelial cell as is seen in lead neuropathy. By contrast, hexachlorophene damages the myelin sheath without affecting the BNB. Edema is strictly confined to the myelin lamellae. In galactose neuropathy the synthesis of a poorly soluble macromolecule inside the BNB changes the local osmotic force causing fluid retention and elevated EFP. Other changes for which are the result of perturbations in all these mechanisms. Additional analysis of transverse sections in lead neuropathy suggest that the perineurial sheath is a compliance which is analagous to brain. As the edema fluid begins to accumulate, transfascicular area (TPA) increases but EFP decreases. Continued expansion of endoneurial volume is restricted by the perineurium which begins to stretch. This is associated with a rise in EFP. At approximately 10 cm H2O, EFP peaks but TFA continues to increase. This may be explained by proximo-distal transport of endoneurial fluid which is augmented by local EFP.

Supported in part by the Veterans Administration and the American Heart Association, California Affiliate.

INCREASED AXONAL PROTEOLYSIS IN MYELIN-DEFICIENT MUTANT MICE.

R.A. Nixon. Ralph Lowell Laboratories, Harvard Medical School and McLean Hospital, Belmont, MA 02178.

Neurons appear to play a critical role in mediating the formation of myelin. In this study, we found specific alterations of axonal proteolysis in three mutant strains of mice which exhibit CNS hypomyelination. The degradation of axonal proteins was measured within a single neuron type for each strain, and axonal proteolysis in three mutant strains of mice which exhibit CNS hypomyelination. In this study, we found specific alterations of axonal proteolysis in three mutant strains of mice which exhibit CNS hypomyelination. The degradation of axonal proteins was measured within a single neuron type for each strain, and axonal proteolysis was increased 260% at pH 3.8 (p<0.001). The different abnormality of axonal protein degradation was elevated 300% at pH 7.4 (p<0.001) compared to the rates in littermate controls. Axonal proteolysis is increased in mutant mice but TFA continues to increase. This may be explained by proximo-distal transport of endoneurial fluid which is augmented by local EFP.

Supported in part by the Veterans Administration and the American Heart Association, California Affiliate.

A NEW HEAD INJURY MODEL FOR EVALUATION OF TREATMENT MODALITIES.


The evaluation of the effectiveness of various treatment modalities in head injury has been hampered by the lack of an appropriate animal model exhibiting extended coma and delayed death. We have developed an animal model that utilizes a repetitive acceleration plus deceleration injury to the intact brain of the anesthetized cat (1400 positive and negative 80-85g impulses applied over 67 seconds) followed after 40 minutes by a one hour period of controlled respiration with 63% O2 in N2 which results in an arterial pO2 of 24 ± 4 torr. This results in delayed death in approximately 50% of animals with an impaired rate of neurological recovery only exceeding the rate if surviving animals are given a numerically quantifiable neurologic examination at regular intervals up to 24 hours and all animals undergo autopsy by examination at approximately 10% of animals die early following the trauma alone and thus are not usable in evaluation of treatment modalities. Control studies evaluating anesthetics alone compared with anesthesia plus hypoxia show no mortality or morbidity from the hypoxia alone. Individual dose response characteristics for variation in duration of hypoxia, magnitude of hypoxia, and time to hypoxia show an acceleration of recovery in many survivors. All surviving animals were increased 260% at pH 3.8 (p<0.001). The different abnormality of axonal protein degradation was elevated 300% at pH 7.4 (p<0.001) compared to the rates in littermate controls. Axonal proteolysis is increased in mutant mice but TFA continues to increase. This may be explained by proximo-distal transport of endoneurial fluid which is augmented by local EFP.

Supported in part by NIH grant NS3042.

STUDIES OF THE SODIUM AND CALCIUM COMPONENTS OF EVOKED ACTION POTENTIALS (EVAP) CULTURED RAT DORSAL GANGLION NEURONS INFECTED WITH HERPES SIMPLEX VIRUS.

S. George Oakes*, Robert S. Pozos, Richard J. Ziegler*, and Roger W. Percy*. (SPON: E. K. Stastny). Department of Physiology and Neurobiology, University of Minn., Duluth, School of Medicine, Duluth, MN 55812.

Disssociated neuron cultures from rat dorsal root ganglia were maintained for 4-6 weeks. Normal electrophysiological parameters were established. Axonal protein degradation was measured in vitro with p-14C-proline. After 5 days, intact optic nerves contained 30% more labeled amino acids at pH 7.4 (Dunlop & Lajtha, 1975, Brain Res. 34:333). Protein synthesis inhibitors were added to prevent reutilization of amino acid pool. The degradation of axonal proteins was normal at pH 7.4 (112%, n=16) but was increased 260% at pH 3.8 (p<0.001) compared to the rates in littermate controls. Axonal proteolysis is increased in mutant mice but TFA continues to increase. This may be explained by proximo-distal transport of endoneurial fluid which is augmented by local EFP.

Supported in part by the Veterans Administration and the American Heart Association, California Affiliate.
"CYTOPLASMIC BODIES" IN HUMAN SKELETAL MUSCLE CO-CULTURED WITH FETAL MOUSE SPINAL CORD COMPLEX, Edith R. Peterson, Edmund B. Neufeld, Alfred Solp*, and Stanley J. Greif, Dept. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Human skeletal muscle innervated by co-cultured explants of fetal mouse spinal cord complex leads to greatly enhanced differentiation of many regenerated muscle fibers (Pet. & C., Exper. Neurol. 72). "Cytoplasmic bodies" (MCB) were developed abundantly (100-500 per culture) in muscle explanted from a biopsy of Duchenne dystrophy (9-month child). Many of these "bodies" were observed in cultures of normal cells in cross-striated areas of cross striations. In the living cultures, birefringent discernible by high-power light microscopy in the living cultures distended regions towards the ends of both undifferentiated or halo -- not membrane-bounded. The bodies are most numerous in ends of some muscle fibers, and to a lesser degree along intercalated disks which merges with the surrounding halo composed primarily of myofilament lattices. Thicker (~10-30 nm) filaments are veal fine-structural features consonant with our light microscopic study of such sites where the MCBs were observed. Transmission electron microscopy of these bodies in 7-16 week cultures revealed fine-structure characteristically different from both normal muscle and with EM studies of certain myopathies in situ (McDonald and A. Engel, Acta Neuropath. 69). The central core consists essentially of a dense tangle of ~3-6 nm filaments, which merges with the surrounding halo composed primarily of radially arrayed ~5-7 nm filaments that may dovetail with near Tenovski (lamellar --- 0.5-3 nm) filaments. The MCBs are seen at times in this zone which otherwise excludes such organelles as mitochondria, endoplasmic reticulum and tracts.

7.0 µm in diameter and were distributed randomly on the endo-vascular surface of the adrenal gland. The MCB bodies were found to contain large numbers of MCBs in greatly enlarged cell somas and were observed at times in cross sections of various organ systems and in the peripheral nervous system. The MCB bodies are not membrane-bounded. The MCBs are by electron microscopy in cross-sections of the human skeletal muscle innervated by co-cultured explants of fetal mouse spinal cord complex and in areas of cross striations. The MCBs are by electron microscopy in cross-sections of the human skeletal muscle innervated by co-cultured explants of fetal mouse spinal cord complex. Electron microscopy of these bodies in 7-16 week cultures revealed fine-structure characteristically different from both normal muscle and with EM studies of certain myopathies in situ (McDonald and A. Engel, Acta Neuropath. 69). The central core consists essentially of a dense tangle of ~3-6 nm filaments, which merges with the surrounding halo composed primarily of radially arrayed ~5-7 nm filaments that may dovetail with near Tenovski (lamellar --- 0.5-3 nm) filaments. The MCBs are seen at times in this zone which otherwise excludes such organelles as mitochondria, endoplasmic reticulum and tracts.

VIRUS REPLICATION: AN EPIPHENOMENON OF THE AXON REACTION?


The present investigation was undertaken to determine, if within the cerebral vasculature altered endothelial morphology alters virus infection or replication. To this end 40 cats having received an IV injection of horse-radish peroxidase (HRP) were subjected to either mechanical brain injury or a 20-min period of systemic hypotension induced by angiotension. Following a brief survival the animals were perfused with aldehydes and their brains were sectioned on a vibratome. Serial sections were then treated with cytochemical and immunohistochemical reagents and the alternate serials were processed for electron microscopy. Light microscopy demonstrated that various loci throughout the neoplasms displayed peroxidase exudation. TEM examination of such sites revealed that the vasculature possessed endothelium displaying numerous HRP laden vacuoles, vesicles and tubules. Serial sections suggested that these HRP containing elements participate in the sequestration and transport of virus in the human community, to reactivated productive infection.

These studies suggest that among the metabolic alterations associated with viral infections of the central nervous system, we also have examined the functional status of cortical neurons in feline mutants with confirmed GM, or GM2-gangliosidoses. Micropipettes for intracellular recording were filled with HRP for subsequent identification of impaled neurons. Cells encountered in the motor cortex were examined for antidromic (cerebral peduncle/pyramidal tract) or orthodromic (thalamic-VL) stimulation. Neurons impaled in feline mutants at various stages of clinical neurobehavioral deterioration exhibited membrane potentials and evoked PSP sequences qualitatively similar to those observed in normal animals of comparable ages. Although EPS-positive discharge were present in the unlesioned CNS of all mutants, there was no absolute selectivity of the evoked discharge over those observed in normal cats. The types of HRP-filled cells were recovered from prepara-
DOSE (mg/kg)

| 15 | 50 | 0 | 83 | 24 | 4 | 0 | 15 | 50 | 84 | 92 | 72 | 50 | 84 | 15 |

The occurrence of ephapses after peripheral nerve injury lends support to longstanding hypotheses concerning their formation, such as the staggerer hypothesis (Zeev J. Seltzer* and Marshall Devor (SPON: Donald Price). Neurology, 1978). We found that the number of neurons in sg dcn is not markedly different from that in normal dcn. However, the staggerer mutant neurons are atrophic; cross-sectional area of representative sections revealed reduction in cell body size of all populations of neurons. The diminished size of neurons in sg dcn is also caused by a reduction in the areas occupied by both the nuclei themselves and the white matter surrounding them.

We conclude that the sg gene does not act on those derivates of cells from the ventricular germinal layers which become Purkinje and Golgi II cells and, 2) transneuronal degeneration does not take place following the formation of a neuroma. The strongest arguments are: 1) in the early end to end resuture or after sciatic crush. In each of these, however, decay within minutes after the injury site. These, however, decay within minutes after the lesion. In contrast to this acute phenomenon, we found that starting 30 days after the lesion, persistent electrical cross-talk between pairs of axons can be detected. In adult rats we cut the sciatic nerve and prevented its regeneration, promoting the formation of a neuroma. As in the foregoing preparations, we severed the L 4-6 dorsal or ventral roots and delivered electrical stimuli (1.5 msec, 1.5 Hz, up to 4 mA) to the ipsilateral portion of the sciatic nerve. In all animals that survived 30 days after the lesion, we recorded from ventral or dorsal root straddles that were cut centrally. Each action potential was recorded at the same threshold latency (1.3 ± 0.2 msec) and the possibility that we were stimulating and recording from one continuous axon possessing a long recurrent sprout, can be ruled out for at least 75% of the fibers recorded at 30-35 days after the lesion, on the basis of response latency. There is, therefore, cross-talk between independent axons. The interaction must occur in the vicinity of the injury since local anesthetic blockade or section of the nerve just proximal to the neuroma eliminates it. Such interactions were found to exist between pairs of axons in each recorded strand. The mean fraction of these being epaphatically connected to sensory fibers was 6.8%. This interaction is probably electrical and not chemical, as it is bilateral and has a very high safety factor (absolute refractoriness as determined by the double pulse method at as little as 0.55 msec; some follow tetanus to 1000 Hz). Based on collisions of action potentials initiated at the dorsal root or at the level of the ventral root just proximal to the neuroma, we could calculate the conduction velocities of the sensory and motor axons involved in each ephapse. Their conduction velocities, obtained after cutting or after the distal segment of the nerve was left stump, did not differ much from the values used in the analysis of the distribution of dorsal and ventral root fibers in a neuroma. We repeated some of these experiments in rats that underwent sciatic section and immediate end to end anastomosis or after the distal end of the nerve was left as a stump.

This study was supported by a grant from the National Multiple Sclerosis Society.
ALTERATIONS IN MAZE PERFORMANCE ASSOCIATED WITH CHRONIC IMMUNE COMPLEX DISEASE. David N. Shucard, Steven A. Hoffman, Ronald J. Harbeck*, Andrew A. Hoffman*, and Hilary A. Krolewski. Dept. of Behavioral Sciences and Dept. of Medicine, National Jewish Hospital and Research Center, Denver, CO 80206.

Evidence is accumulating to suggest that immune complexes may be responsible for altering central nervous system (CNS) function in individuals with systemic lupus erythematosus (SLE). Studies from our laboratories and others, using animal models, have shown an array of CNS-associated effects of laboratory-induced immune complex disease. These include immune complex deposition in the choroid plexus, changes in cerebrospinal fluid composition, and effects on behavior. In this investigation a rat model was used to study the effects of chronic immune complex disease on CNS functioning and on subsequent maze learning performance. Chronic immune complex disease was induced in rats by 3 initial subcutaneous injections of bovine serum albumin in Freund's incomplete adjuvant followed by bi-weekly or tri-weekly intravenous injections of bovine serum albumin. Behavioral measures of speed, errors and trials to criterion in a Lashley Maze were obtained. Proteinuria used as an index of the disease process was assessed over the course of the experiment. At the end of the study, the presence of immune complex deposits in the kidney and choroid plexus was determined by immunofluorescence microscopy. The results indicated that there were differences in maze performance associated with proteinuria levels. Experimental animals with elevated proteinuria produced significantly less errors and had fewer trials to criterion than either the experimental animals with low proteinuria or the controls. These same findings were obtained when animals were retested in the maze after a two week interval. Immunofluorescence data showed that none of the control animals but a majority of experimental animals showed evidence of immune complex deposits in the kidney and choroid plexus. In general, choroid plexus immunofluorescence was of lower intensity and more variable than that seen in the renal glomeruli. Furthermore, the data indicated that immune complex deposits in the choroid plexus may be related to performance in the maze. These results confirm and extend previous data from our laboratories reporting associations between the induction of chronic immune complex disease in rats. The types of behavioral alterations observed may be related to changes in attention-related mechanisms in the CNS. (Supported in part by USPHS Grant NS-12594.)


Electrically induced hippocampal (HPC) after-discharges (ADs) and their sequelae have been recommended as an index of neuropsychiatric dysfunction which to measure toxicant-induced change. The properties of AD (ADs) have been characterized both under normal conditions and following exposure to a number of toxicants. The present experiment was designed to assess the effects of acute systemic ethanol exposure upon these afterdischarge (AD) thresholds. Thirty male hooded rats were bilaterally implanted with bipolar stimulating and recording electrodes in the dorsal HPC. On test days animals were injected with either 0, 0.125, 0.25, 0.50, 2.0, or 3.0 g/kg of ethanol (IP) in a 20% (v/v) solution. Thirty minutes later AD thresholds were determined by a method similar to that described by Finel et al. (1976). Each animal received each dose once in a counterbalanced order. Tail vein blood samples were taken immediately after the AD and assayed to determine blood-ethanol concentration. The most clear and profound effects were observed with the 3.0 g/kg dosage. Blood-ethanol concentrations at this dosage typically fell between 212 and 305 mg/dl. At this dosage the threshold for AD production was elevated, AD duration was decreased, and the frequency of spikes within ADs was decreased. These results parallel those obtained in previous investigations concerning sedative-adverse alterations in HPC ADs and their sequelae, and suggest alterations in HPC ADs as an index of alcohol-induced neurophysiological depression. The results will be discussed in light of a dose response study on ethanol-induced changes in single unit activity in the hippocampus (Neuropharmac. 18: 61, 1979).

MEMBRANE ELECTRICAL EVENTS ASSOCIATED WITH LYMHPHOCYTE KILLING OF CULTURED NERVE CELLS. Cathy L. Stephanos, Sandra Fitzgerald, and Pierre A. Henkart*. NCI & NICHHD, NIH, Bethesda, MD 20205.

Intracellular microelectrode recordings were made of neuroblastoma-glioma hybrids (NG 8-2) which were attacked by thymus derived (T) killer lymphocytes. Spleen cells from C57 B1/6 strain (H-2b) were sensitized in vitro in a standard 5-day culture against irradiated BIO.A or AJ5 (H-2d) mice in order to generate killer T cells which would recognize H-2d, (the haplotype from which the neuroblastoma-glioma target cells were derived). Using the standard 51Cr release assay with the neuroblastoma-glioma target cells, lysis was observed with such b anti-a lymphocytes but not with similarly sensitized a anti-b lymphocytes or with b lymphocytes co-cultured with irradiated b haplotype cells. Several lines of evidence indicate that killer T-lymphocytes induce channels in the target cell's membrane which allow ions to equilibrate across the membrane leading to colloid osmotic lysis. To investigate the membrane electrical events associated with this killing, intracellular microelectrode recordings were made in a neuroblastoma-glioma cell while adding activated T-lymphocytes and until the cell either completely lost its membrane potential or until the electrode was withdrawn after a long term recording (up to 4 hrs.). Prior to any treatment the resting potential of these cells was ~60 mV and the cells were typically electrically silent, but produced action potentials following intracellular stimulation. After lymphocytes were layered over the target cells, large reversible depolarizations (20-40 mV) lasting from 3-30 sec were seen in the target cells. Between intermittent depolarizations the resting membrane potential remained constant.

Although all recorded cells maintained a normal resting membrane potential over a long period of time, the data suggested, later (78-140 mins) 27% of these cells irreversibly lost their membrane potential following an abrupt depolarization from ~60 to 0 mV. All of the electrical changes described were seen using H-2d anti-2g-lymphocytes; the control lymphocytes which did not show 51Cr release from these target cells did not cause significant electrical changes in the target cell membrane. Our results suggest that while T-lymphocytes initially may transiently increase membrane permeability, progressive conic equilibration following the interaction of target cells and T-lymphocytes does not seem to occur.

ALUMINUM ENCEPHALOPATHY—INHIBITION OF HXK2 DEGRADATION BY ALUMINUM. George A. Trapp* (SPIN: F.C. Jobe). Research Service, Veterans Administration Hospital and Dept. Psychiatry, LSU School of Medicine, Shreveport, LA.

The aluminum encephalopathy model of aluminum neurotoxicity and neurofibrillary degeneration (NFD) has been studied in experimental animals and may have some relationship to human renal dialysis encephalopathy and also to a variety of conditions lead to NFD, including Alzheimer's Disease. In the absence of specific information on aluminum function, we considered the possibility that aluminum exerts toxic effects by interference with well-known metal-activated reactions. We selected magnesium-dependent phosphoryl transfer catalyzed by yeast hexokinase as a convenient example for study. Aluminum-ATP complex gives kinetics of linear competitive inhibition against magnesium-ATP as the varied substrate (Km = 8 x 10^-6 M, KI = 4 x 10^-6 M), and is non-competitive against glucose (Km = 3 x 10^-6 M). Aluminum-ATP was not a phosphoryl donor in this reaction and velocity is zero in absence of magnesium. The stability constant (Ks) for aluminum-ATP was 7.4 x 10^-6 M and for magnesium-ATP was 1.2 x 10^-6 M. Chromium-ATP, a known inhibitor of HK had Ks = 5.5 x 10^-6 M and gallium-ATP had Ks of 2.3 x 10^-6 M.

We conclude that aluminum ion is a dead-end inhibitor of hexokinase. KI is in the range of aluminum concentrations seen in experimental animal models of aluminum toxicity in brains. Inhibition of this or some other magnesium-ATP enzyme may be an important step in the genesis of NFD in animals or dialysis encephalopathy in man.

Supported by the Medical Research Service of the Veterans Administration.
1753 DEVELOPMENT OF ABNORMAL NEURON MORPHOLOGY IN FELINE GANGLIOSIDOSIS


Recent studies strongly suggest that the pathological biology of ganglioside storage disease is intimately linked to progressive changes in neuron shape and synaptic distribution rather than to simple cytotoxic effects of ganglioside accumulation (Brain Res. 116: 1-21, 1976). Of primary interest is the formation of abnormalities termed meganeurites, which are enlargements at the junctions of neurons and axon terminals. Their synaptic contacts have unknown origin. Preliminary studies of feline GM1 gangliosidosis have revealed similar morphological changes present in various types of neurons seen in GM1 CNS (Brain Res. 116: 13-26, 1977).

The availability of colony-reared cats with GM, and more recently GM2 gangliosidosis, which bear remarkable similarity to the disorders seen in children (Science 183:839, 1971; Science 196: 1014-1017, 1977), has made possible a systematic study of the development of these morphological changes in the feline CNS during progression of the disease. Although the specific types of neuronal alterations are similar in these two types of feline gangliosidosis, the neurobehavioral deterioration progresses at different rates. The availability of colony-reared cats, are seen at 6-8 weeks of age in GM, and 12-15 weeks in those with GM2, gangliosidosis. Similarly, onset of recumbency is more rapid in GM cats, occurring at 4 months as opposed to 6-7 months in GM2 mutants. Terminal stages are reached by 6 months in GM, 12 months in GM2, cats.

Progressive morphological alterations can be demonstrated in Golgi preparations of neurons during the course of clinically evident neurological deterioration. Changes in individual neuronal types and type-specific neuronal types (e.g. cerebellum) do not demonstrate any abnormalities. Examples of affected neurons include pyramidal cells of cerebral cortex, medium spiny cells of caudate and putamen, apparent intrinsic neurons of thalamic and reticular nuclei, and stellate cells of cerebellum. Some of the alterations occurring in particular neurons include simple enlargement of the soma (progresive), per-membrane structure progressing to degeneration, variable focal enlargements in dentritic trees, and secondary neurite formation, primarily from abberant sites such as meganeurites. Changes seen in any one neuron type are both specific and consistent, however, especially in pre-terminal stages, apparent normal neurons of a particular kind can be demonstrated in proximity to those showing morphological abnormalities. (Supported by NS-07512 and NS-10967.)
NEUROPEPTIDES
NALOXONE ALTERS LOCOMOTION AND INTERACTION WITH ENVIRONMENTAL STIMULI. Amy J. Armenta* and David S. Segal. Dept. Psychiatry, Sch. Med., UTSouthwestern Med Ctr., Dallas, TX 75235.

Effects of the opiate antagonist naloxone were examined on the behavioral response pattern of rats in a novel environment. After injection with saline or naloxone (0.5, 5.0 or 25 mg/kg, s.c.), the rats were monitored for locomotion and both frequency and duration of contact with stimuli in a multicompartment exploratory chamber. Naloxone produced a dose-related reduction in locomotion and in frequency of contact with stimuli. At the lowest dose tested this reduction was accompanied by an increase in total duration of contact and in time spent per contact with the stimuli. In contrast, the highest dose of naloxone decreased the duration of contact with stimuli and induced prolonged periods of inactivity. An intermediate response was observed with 5.0 mg/kg naloxone. These findings indicate that lower doses of naloxone may enhance interaction with environmental stimuli while the predominant effect of higher doses is a general suppression in behavioral activity.


Immunoreactive α-MSH (α-MSH) (Oliver and Porter, Endocrinology 102: 697, 1978) and ACTH (ACTH) (Krieger et al., Proc Natl Acad Sci 74: 648, 1977) are present in hypothalamic tissue. The current view is that in the pars intermedia of the hypophysis of the rat, α-MSH is derived from ACTH, and ACTH in turn is derived from a large (31K) precursor by a process involving a series of cleavage reactions. In addition to α-MSH and ACTH, hypothalamic cells contain significant amounts of the 31K precursor as well as intermediate forms between the 31K precursor and ACTH. We have addressed the question whether the processing of ACTH in hypothalamic tissue may proceed in a manner similar to that operating in the cells of the pars intermedia. We have analyzed the molecular forms of ACTH in extracts of the hypothalamus and pars intermedia and calculated the amounts of the various forms of ACTH and α-MSH, ACTH, and α-MSH, were extracted from hypothalami and neurointermediate lobes of adult male rats with 5M acetic acid (Ellipe and Mais, Biochemistry 14: 3936, 1975). The various forms of ACTH were separated by means of gel filtration using columns of Sephadex G-50 (superfine) under denaturing conditions (5M urea containing 0.5% 2-


We have previously shown that endorphin immunoreactive material appears in the rat brain early in ontogenetic development. This study is an initial attempt to characterize this material. Embryonic day 16 rat brains and pituitaries were extracted in boiling 0.2 M HCl. Extracts from several animals were pooled, concentrated, and lyophilized. The reconstituted samples were analyzed by permeation chromatography and fractions assayed for endorphin immunoreactivity as indicated below.

A single peak, co-eluting with 125I-β-endorphin, was observed in extracts of embryonic brain, indicating that in the embryonic brain as well as in the adult, if any, of the differences in the higher molecular weight endorphin pool is present. In contrast, embryonic pituitary extracts showed a substantial amount of larger endorphin-like material (possibly β-LPH and 31K protein). However, the relative contribution of these peaks to the total pituitary endorphin is much lower than in the adult where β-LPH is the predominant species. This difference might indicate different physiological roles for β-endorphin and β-LPH in prenatal and postnatal life. Currently, we are characterizing the higher molecular weight endorphins in the embryonic pituitary using polyacrylamide gel electrophoresis.

Tyr-DAla-NH₂ has weak, naloxone-reversible, analgesic activity at an intraventricular dose of 100µg (McGregor, Stein & Bellussi, 1979). We report here that, or after chronic administration (5 mg/kg, s.c.), the dipeptide has the opposite effect, producing hyperalgesia and reversal of opiate-and stimulation-induced analgesia of rats. (N = 8). In this experiment, analgesia was induced by morphine sulfate (2.5 mg/kg, s.c.) or the enkephalin analog DAla², DLeu⁵-NH₂, intraventricularly. Administration of the dipeptide 15 minutes after the onset of analgesia significantly shortened the duration of analgesic action of the enkephalin compounds. In a second experiment using a weak heat stimulus, the effects of the dipeptide alone on normal pain sensitivity were examined. Tyr-DAla-NH₂ produced a significant hyperalgesic response compared to controls. (see Figure 1). Naloxone had no effect on hyperalgesia produced by the dipeptide. However, naloxone had no effect on the analgesic activity, nor did it alter the effects of opiate agonists or antagonists. 

EFFECTS OF β-END OR ON BODY TEMPERATURE

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>31º</th>
<th>20º</th>
<th>10º</th>
</tr>
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<tbody>
<tr>
<td>0.03</td>
<td>0.78 ± 0.23</td>
<td>0.96 ± 0.15</td>
<td>0.01 ± 0.16</td>
</tr>
<tr>
<td>0.1</td>
<td>0.90 ± 0.21</td>
<td>1.16 ± 0.10</td>
<td>0.41 ± 0.16</td>
</tr>
<tr>
<td>0.3</td>
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<td>1.30 ± 0.14</td>
<td>0.61 ± 0.17</td>
</tr>
<tr>
<td>1.0</td>
<td>1.48 ± 0.16</td>
<td>1.35 ± 0.23</td>
<td>0.48 ± 0.32</td>
</tr>
<tr>
<td>3.0</td>
<td>1.04 ± 0.16</td>
<td>0.32 ± 0.46</td>
<td>-5.22 ± 0.97</td>
</tr>
<tr>
<td>10.0</td>
<td>1.40 ± 1.14</td>
<td>-5.50 ± 0.75</td>
<td>-14.01 ± 0.54</td>
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</table>

Values shown are the difference in effect between drug treatment and saline control groups at 60 minutes after injection (N=10).

The effect of naloxone (1 mg/kg, s.c.) on both the low dose (0.3 µg) and high dose (10 µg) of β-END was also examined. Pretreatment with naloxone significantly blocked the hyperthermia produced by 10 µg of β-END at 20º C and 10º C. Naloxone did not block the hyperthermia produced by the 10 µg dose of β-END at 31º C (ambient temperature). Similarly, the hyperthermia produced by the 0.3 µg dose of 8-End was not blocked by naloxone at any ambient temperature. These data suggest that β-END may produce its hyperthermic and polikallathic effects at different sites. (Supported in part by USPHS Grant # DA 00124).

DMGRO- AND ENZYME CYTOCHEMISTRY OF NEUROSECRETORY NEURONS IN NORMAL AND SALT-TREATED MICE. R. D. Broadwell, C. Oliver* and M. W. Brightman*. WINDS and NIND, NIH, Bethesda, Md. 20025.

Neurosecretory neurones of the hypothalamo-neurohypophysial system produce and secrete the octapeptides oxytocin and vasopressin. These neurones respond to alterations in osmolarity of the extracellular fluid and osmolality of the zymosome and synthesize these peptides in response to these changes. The perikarya, including their associated Endoplasmic Reticulum from which Lysosomes arise and the Golgi apparatus, were examined ultrastructurally in the nuclear envelope, rough endoplasmic reticulum, cell bodies stain positively for anti-neurophysin, indicating that these cells synthesize the associated octapeptides. PAP activity was confined to GERL and secondary lysosomes. Neurosecretory neurones are present in the lateral hypothalamus, in the arcuate nucleus lesion of which markedly depletes α-MSH concentrations throughout the brain.


An extensive α-MSH containing neuronal system has recently been described in the rat brain (PMS 73, 1968). The perikarya of this system are located primarily in the arcuate nucleus lesion of which markedly depletes α-MSH concentrations throughout the brain.

In an attempt to measure the in vivo release of α-MSH, the lateral ventricle of chloralose-urethane anesthetized rats were cannulated and perfused with artificial cerebrospinal fluid (CSF). The perfusate was collected from an indwelling cannula in the cisterna magna at 20 min intervals. α-MSH concentrations in the perfusate were dependent on both the dose used and the ambient temperature. Doses of β-END between 0.03 and 1.0 µg produce a dose-related hyperthermia at an ambient temperature of 20ª C. At this same ambient temperature, a 10 µg dose produced significant hyperthermia (-6.11º C). In mice studied at 31º C hyperthermia was blocked by all doses (0.03-10.0 µg) tested. At an ambient of 10º C hyperthermia was produced by the lower doses of β-END and hypothenmia by the higher doses (3 and 10 µg). These data demonstrate that the ICV administration of low doses of β-END produce an increase in body temperature whereas higher doses may cause the animal to become polikallathic.


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In human CSF, α-MSH-like immunoreactive compound has also been detected. Concentrations ranged from 4.0 to 73.3 pg/ml with mean of 22.9 pg/ml.

These results suggest that α-MSH present in CSF is not derived primarily from the pituitary gland but may represent neurally released α-MSH.


In pituitary, as well as in hypothalamus, β-endorphin is formed from proopitocytic breakdown of high molecular weight (HMW) protein precursors. The relationship of these precursors to enkephalin is not clear, especially since β-endorphin and enkephalin possess different regional distributions in brain. In order to determine whether other high HMW proteins may be enkephalin precursors, various preparations of rat brain were assayed for reactivity with enkephalin antisera. The antibodies were specific for met- or leu-enkephalin, and did not cross react with β-endorphin. The post-mitochondrial supernatant of rat brain was extensively filtered through Dia-Flo FM-10 membranes and eluted on a Sephacryl S-200 column. Two peaks, HMW >100,000 and HMW 40,000, of enkephalin immunoreactivity were observed. Neither peak non-specifically bound the HMW-enkephalin ligand, nor appeared to bind endogenous enkephalin. The 100,000 HMW peak, however, bound non-specifically to normal rabbit IgG, indicating the presence of an immunoreactive artifact. The 40,000 HMW peak did not bind to normal rabbit IgG and, when prepared from microwave-irradiated brains to which tryptic, the tryptic product did display reactivity in the opiate radioimmune assay in addition to the enkephalin immunoreactivity as determined on a Sephacryl S-200 column. Further analysis revealed that the tryptic product was slightly larger than enkephalin, suggesting that trypsin-like enzymes are not the actual enkephalin-forming enzymes in the brain. However, unlike the high HMW activity, the tryptic product did display reactivity in the opiate radioimmune assay in addition to the enkephalin immunoreactivity as determined on a Sephacryl S-200 column. This technique may prove to be of considerable value in the localization of neuropeptides in the nervous system. Maximum immunoreactivity is retained in the sections, the sensitivity of the method is high and specific for the detection of small terminals in ultrathin sections, and these images can be correlated with electron micrographs having excellent morphology. Supported by USPHS Grants DA-00266 and DA-01645.


The unlabelled antibody-enzyme method of immunocytochemistry was used to visualize LHRH in rat brain. In vibratome sectioned rat and mouse brains, adult male Sprague-Dawley rats and Swiss-Webster mice were fixed by cardiac perfusion of 10% neutral formalin or 4% paraformaldehyde. Brains were decalcified, dehydrated hypothalami indicate that approximately 90% of this high MW activity, the tryptic product did display reactivity in the opiate radioimmune assay in addition to the enkephalin immunoreactivity as determined on a Sephacryl S-200 column. Some of the grids were stained with uranyl magnesium acetate and used only for electron micrography. Other grids were used first for electron micrography, then immunostained for neurophysin and photographed with the electron microscope, yielding images of terminals that could be enlarged and superimposed unambiguously on the electron micrographs. This technique can prove to be of considerable value in the localization of neuropeptides in the nervous system. Maximum immunoreactivity is retained in the sections, the sensitivity of the method is high and specific for the detection of small terminals in ultrathin sections, and these images can be correlated with electron micrographs having excellent morphology. Supported by NSF.


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Mechanically dissociated fetal mouse spinal cord cells were grown in monolayer on collagen-coated glass coverslips. After varying periods of time, dissociated dorsal root ganglion (DRG) cells were added to some cultures. After 2-6 weeks, the cultures were fixed with 4% paraformaldehyde at 4°C for 2 hrs. Fluorescence immunohistochemistry was accomplished using antisera to met-enkephalin (ME), somatostatin (SOM) and substance P (SP). Specificity of staining was established using antisera pretreated with an excess of the appropriate antigen prior to application on cultures. After observation and photography using fluorescence and phase contrast microscopy, the coverslips of cultured neurons were prepared for scanning electron microscopy (SEM) according to standard procedures. Spinal cord cultures exhibited striking networks of varicose fibers, terminals and neuronal perikarya after incubation with ME antiserum. The fibers and terminals were frequently in close apposition to dendritic profiles and perikarya of unstained neurons. Immunofluorescence within neuronal perikarya often revealed an organized intranuclear cytoplasm. The perikarya containing ME immunoreactivity were predominantly multipolar and of small to medium diameter. A large increase in the number of immunoreactive SP and SOM fibers, terminals and perikarya was observed in cultures to which DRG had been added. These fibers and terminals, like those seen with ME antiserum, made frequent contact with unstained dendrites and perikarya. However, many SP and perikarya were smooth, slow and bipolar and were identical to DRG neurons as identified by phase contrast microscopy. Examination of cultures with SOM revealed additional systematic sprouting of the interaction of the varicose fibers and terminals with dendrites and perikarya. The morphological characteristics of the immunohistochemically identified peptides were consistent with the morphology of the same population of peptidergic neurons in situ. Such findings suggest the utility of using cultured neurons as a model of neuronal architecture, connectivity and physiology. (Supported in part by McNair Scholars Award to R.E.).

EXCITATORY ACTION OF OPIOID PEPTIDES ON CULTURED HIPPOCAMPAL PYRAMIDAL CELLS. B.R. Gehlweiler. Pharmaceutical Division, Preclinical Research, SANDOZ Ltd., CH-4002 Basle, Switzerland.

Spontaneous activity was recorded intracellularly from rat hippocampal pyramidal cells maintained in explant cultures for several weeks. Pyramidal cells were recognized morphologically by their size and by their characteristic pattern of dendritic arborization following injection of horseradish peroxidase into single neurons. Bath application of opioids or opioid peptides rapidly increased the amplitude of epsp's until large depolarization shifts and spike bursts occurred. Using the stable methionine enkephalin analogue, FK 33-824, excitatory effects were observed at concentrations as low as 10-8M. The effects were dose-dependent; high concentrations of FK caused sustained depolarization of the neurones. Identical results were obtained with morphine (10-6 to 10-4M), met-enkephalin (10-7M) and leu-enkephalin (10-7M). The effects of all these substances were shown to be reversible and naloxone-sensitive. Our findings that these peptide effects were mimicked by blockade of GABA receptors with bicuculline methochloride and abolished by synaptic isolation with 8 mM Mg2+ suggest that the excitatory action of opioid peptides on hippocampal pyramidal cells comes about by removal of tonic gabaergic inhibition by basket cell interneurons (also Siggins et al., Soc. of Neuroscience, 8th Annual Meeting, St. Louis, 1978). Since iontophoretic application of enkephalin directly onto pyramidal cells in control solution unexpectedly produced similar excitatory effects, the possibility is raised that enkephalin receptors are located presynaptically on pyramidal cell afferent terminals.


Recent studies have suggested that somatostatin (SRIF) is contained in both neuropeptide and non-neuropeptide neurons and may function as a neurotransmitter as well as a neurohormone. We have undertaken a study of the distribution and morphology of SRIF-containing neurons in the guinea pig forebrain. SRIF was localized in 6 μm paraffin sections by the unlabeled antibody enzyme technique of Sternberger using an antisera to SRIF. Following immunocytochemical identification of SRIF, sections were counterstained in cresyl violet. Perikaryal diameter was determined by measuring the long and short axes using a 40× objective and 12.5 × ocular equipped with a measuring reticule. SRIF-containing neurons were determined in the following areas (range and (mean)): Hypothalamus: anterior hypothalamus, 10-19 μm (13.6μm); medial nucleus, 10-12 μm (11.2 μm). Hippocampus: oriens layer, 11-18.5 μm (13.6 μm); molecular layer, 10-13.5 μm (11.4 μm); pyramidal layer (CA1-CA3), 11-12.5 μm (11.8 μm); Hypothalamus: anterior hypothalamus, 10-19 μm (14.2 μm); lateral hypothalamus, 10-12 μm (11.2 μm). Ventromedial nucleus, 10-12 μm (11.2 μm). In many cells of the forebrain SRIF was present in thick, unbranched processes, presumably dendrites, and in thin, beaded fibers which could be axons. SRIF-containing neurons in the neocortex appeared round or oval. The diameters of SRIF-containing neurons were determined in the following layers: CA1-CA4, 11-12.5 μm (12.3 μm); Nucleus of the diagonal band: 8.5-15.5 μm (12.3 μm); Accumbens: 10-19 μm (15.5 μm); Amygdala: 15-25 μm (18.5 μm); Paraventricular thalamus, 10-12 μm (11.2 μm). The fibers and terminals were frequently in close apposition to dendrites and perikarya. Immunofluorescence was restricted to a narrow zone of perinuclear cytoplasm. The perikarya containing ME immunoreactivity were predominantly multipolar and of small to medium diameter. A large increase in the number of immunoreactive SP and SOM fibers, terminals and perikarya was observed in cultures to which DRG had been added. These fibers and terminals, like those seen with ME antiserum, made frequent contact with unstained dendrites and perikarya. However, many SP and perikarya were smooth, slow and bipolar and were identical to DRG neurons as identified by phase contrast microscopy. Examination of cultures with SOM revealed additional systematic sprouting of the interaction of the varicose fibers and terminals with dendrites and perikarya. The morphological characteristics of the immunohistochemically identified peptides were consistent with the morphology of the same population of peptidergic neurons in situ. Such findings suggest the utility of using cultured neurons as a model of neuronal architecture, connectivity and physiology. (Supported in part by McNair Scholars Award to R.E.).

SOMATOSTATIN IN RAT CORTICAL NEURONS IN CELL CULTURE: SYNTHESIS AND PHYSIOLOGIC EFFECTS. John Delft*, Marc Dichter, Richard Robbins*, James Connolly* and Seymour Rechlin* (SPON. Howard Blum). Dept. Neurology, Children's Hospital Medical Center, Boston, MA 02115.

Rad cortical neurons, maintained in a dissociated cell culture system have been assayed for various neuropeptides. The cultures contained somatostatin (SS) and substance P (SP) activity. Activity of both substances was determined by measuring the long and short axes using a 40× objective and 12.5 × ocular equipped with a measuring reticule. SS concentration of both cells and media went from undectable at 0 to 5 days to nanogram levels after 3 weeks in culture. Immunofluorescence was present only in neurons and in no other cellular elements. Approximately 5% of the neurons contained somatostatin. Staining was prevented by preadsorption of the anti-SS antibody with synthetic cyclic SS. SS-containing neurons had no definite morphologic appearance. Direct application of SS by microperfusion (0.1 to 10 μM) to cortical neurons in these cultures produced no significant change in resting membrane potential, membrane resistance, or input resistance. SRIF neurons in the hippocampus: oriens layer, 11-18.5 μm (13.6 μm); molecular layer, 10-13.5 μm (11.4 μm); pyramidal layer (CA1-CA4), 11-12.5 μm (11.8 μm); Hypothalamus: anterior hypothalamus, 10-19 μm (12.3 μm); lateral hypothalamus, 10-12 μm (11.2 μm); Ventromedial nucleus, 10-12 μm (11.2 μm). In many cells of the forebrain SRIF was present in thick, unbranched processes, presumably dendrites, and in thin, beaded fibers which could be axons. SRIF-containing neurons in the neocortex appeared round or oval. Most of these cells had only one immunoreactive process, whereas those in the anterior hypothalamus-periventricular zone had one to several immunoreactive processes. SRIF neurons in the ventromedial nucleus tended to be small with one or none immunoreactive processes. Our results suggest that SRIF is synthesized in several different types of neurons; the functional implications of this remain to be determined. Supported by HD5645 to A.J. Silverman and Postdoctoral Training Grant I-TK-32 GM07061 to S.C. Feldman.
The effects of various opiate agonists and an antagonist on the amino acid responses of spinal neurons was studied using cultured, dissociated, fetal mouse spinal neurons and conventional electrophysiological techniques. Intracellular recordings were made from large (25 to 50 µm) neurons which had been maintained in culture for 1 month or longer. The recording media consisted of Hank's BSS plus Hepes buffer (25 mM) and 10 mM MgCl2. The putative amino acid neurotransmitters glycine (GLY), γ-amino butyric acid (GABA), α-alanine (α-ALA) and glutamate (GLU) were applied extracellularly by iontophoresis. The opiate agonists and antagonist were applied by superfusion. All effects were dose-dependent and reversible. When applied at 10 to 100 µM concentrations, the opiate agonist levorphanol (LP) depressed the inhibitory responses evoked by GLY and α-ALA but did not affect the inhibitory GABA response or the excitatory glutamate response. Dextrorphan (DP), the stereoisomer of LP, was ineffective or only slightly depressed the GLY response at these concentrations (effect on α-ALA not tested). Morphine (50 µM), another opiate agonist, also depressed the inhibitory GLY and α-ALA responses without altering the GABA response (effect on GLU not tested). The ability of naloxone (NAL), an opiate antagonist, to reverse the LP and morphine depressions is presently being tested to determine if these effects are mediated by opiate receptors. Leucine-enkephalin, an endogenous opioid peptide believed to act at opiate receptors, did not affect the amino acid responses of these neurons although such modulatory effects of leucine-enkephalin have been observed in other cultured spinal neurons. (+)-NAL, the form of NAL which is active at opiate receptors, depressed the inhibitory GABA response when applied at 0.1 to 1 mM concentrations but did not depress the GLU response. The GLY response was slightly depressed at the higher (+)-NAL concentrations. This effect of (+)-NAL was not stereospecific since (+)-NAL, the stereoisomer of (+)-NAL, also blocked the GABA response. (-)-NAL did not alter the GABA or GLU response when applied at 10 to 100 µM concentrations but completely blocked the GLY response. The effect of (-)-NAL on GABP, dose-response curves suggests that (+)-NAL acts as a competitive antagonist at the GABA receptor but the mechanism underlying the (+)-NAL depression of the GLY response has yet to be determined.

The strain difference in brain Met-ENK may, however, be involved in other established behavioral differences between C and D mice (Oliveiro et al., Adv. Biochem. Psychopharmacol. 11, 411, 1974).

Data from both strains do not support a correlation between whole brain ENK levels and any of the strain differences in behavior noted above. The strain difference in brain Met-ENK may, however, be involved in other established behavioral differences between C and D mice (Oliveiro et al., Adv. Biochem. Psychopharmacol. 11, 411, 1974).
1779 β-ENDORPHIN INDUCED EPILEPTIFORM ACTIVITY INCREASES LOCAL CEREBRAL METABOLISM IN HIPPOCAMPUS, ANTYDALA AND SEPTUM. Steven J. Henniker, Frederic Morrison, and Floyd L. Bloom. A.V. Davis Ctr., The Salk Inst., La Jolla, CA 92037.

The non-convulsive limbic EEG epileptiform activity elicited by intracerebroventricularly administered β-endorphin (Reiziss et al. PNAS 75 (10) 5221-5225, 1978) was metabolically mapped using the 14C-2-deoxyglucose technique (Sokoloff et al 1977, J. Neurochem. 28, 8, correlated with the histologically labeled, morphologically identified, both striate and the lateral ventricle through an implanted guide tube. Injection of a dye solution ivt at the end of 20 min. The effects of saline injections were compared to subse­quent naloxone HCl (48 µg in 20 µl saline) restored MAP and pulse pres­sure (PP) to control values within 5 min; no significant effect on heart rate was observed. Respiratory rates and colonic tempera­ture which were depressed by spinal transection, were significant­ly increased by spinal cord at C6-C7 in anesthetized male rats weighing 500-800 g.

Our results provide evidence that the decline in respiratory movements may be due to the effects of β-endorphin on the respiratory center. The results of these experiments suggest that β-endorphin may have a role in the control of respiration through the modulation of neuroendocrine and neurotransmitter systems.
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1781

An extensive system of $\alpha$-MSH-containing nerves which originates primarily from the arcuate nucleus of the hypothalamus and projects to many forebrain, midbrain and some hindbrain sites has recently been described. A common feature of neurotransmitters is a depolarization-induced release mechanism. In this study, such a mechanism was investigated using an in vitro approach. Fresh rat hypothalami were sliced (approximately 225 µm) using a tissue chopper. Sections were suspended in 0.6 ml of a modified Krebs-bicarbonate buffer which was maintained at pH 7.4-7.6. $\alpha$-MSH released into the medium was analyzed by radioimmunoassay. High concentrations of $\alpha$-MSH diffused from the slices into the medium immediately upon incubation. Rinsing the slices with buffer reduced the baseline release to 31 pg/2 minutes. Stimulation with K+ (50 mM) resulted in a marked increase (250%) in $\alpha$-MSH release. K+ stimulation in the absence of calcium (1 mM EGTA) also resulted in $\alpha$-MSH release but was significantly attenuated compared to K+ stimulated release in the presence of Ca2+ (5 mM). Veratridine (100 µM) also markedly stimulated $\alpha$-MSH release (300%). Once again this release was significantly depressed in the absence of Ca2+. These results suggest an $\alpha$-MSH release process and support a neuroregulatory role for this peptide.

1782

Unilateral intrahippocampal injection of kainic acid (K.A.) (1 µg in 1 µl of 0.9% NaCl) produced recurrent motor seizures lasting about 5 hr and a selective change in the [met]enkephalin (ME) content of hippocampus. Six to 12 hr after injection, there was a small decrease in ME content in both injected and contralateral hippocampus, however within 24 hr after the injection the ME content was increased. The elevation in the injected hippocampus reached a maximal level (200%) after 2 or 3 days, this plateau lasted longer than 14 days. In contrast the ME content of the contralateral side increased by a greater extent reaching a peak at day 2 (300%) but returned to control level by day 14. The increase in ME content was greatly reduced when cycloheximide was administered intraventricularly 6 hr after intrahippocampal injection of K.A. This result suggests that the increase in ME content elicited by K.A. injection is probably due to an increase in the synthesis of ME rather than to a decrease in the efflux of ME from the hippocampus. The selective increase in hippocampal ME content was also elicited by intraperitoneal or intravenous injections of K.A. Since all the procedures caused recurrent motor seizures it is suggested that the change in hippocampal ME content after K.A. may be related to the recurrent seizures elicited by K.A. In fact the increase in ME content after intraperitoneal injection of K.A. was blocked when muscimol, an anticonvulsant, was injected together with K.A. Moreover, the increase in hippocampal ME 2 days after recurrent seizures was also induced by isoniazid or by repeated electroconvulsive shock (5 shocks given at 20 min intervals). Thus, it appears that prolonged recurrent seizures may cause a selective increase in the production of hippocampal ME.

1783

The tricyclic antidepressants and cocaine are potent inhibitors of the synaptosomal reuptake of norepinephrine. In addition, the tricyclic antidepressants are potent antagonists of muscarinic cholinergic, α-noradrenergic and histamine receptors. These interactions may explain the therapeutic actions and side effects of these drugs. We have studied the binding of 3H-imipramine (3H-IMI), 3H-desipramine (3H-DMI) and 3H-cocaine to rat brain membranes. The binding of 3H-IMI to crude brain membrane fractions is saturable, and subcellular fractionation suggests a synaptosomal localization of the 3H-IMI binding site(s). Imipramine (10^-7M) displaces 60-70% of total bound counts with an IC50 of approximately 80 nM. Scatchard analysis indicates binding to at least two separate sites with affinities of 4 and 20 nM. The detailed pharmacologic profile of 3H-IMI binding will be described, and the possible interaction with the binding of 3H-phenylcyclidine (3H-PCP) will be discussed.

In order to investigate the mechanism of inhibition of norepinephrine uptake by the tricyclic antidepressants and cocaine, we have studied the synaptosomal uptake of 3H-IMI and 3H-cocaine. The uptake of both of these ligands appears dependent on time, temperature, and the integrity of the synaptosomal preparation. The data suggest that imipramine and cocaine inhibit the uptake of norepinephrine by being taken up themselves into synaptosomes. The detailed pharmacologic and kinetic parameters of synaptosomal uptake of 3H-IMI and 3H-cocaine will be described.

1784
A PEPTIDE RESEMBLING LUTEINIZING HORMONE RELEASING HORMONE AS A LIKELY TRANSMITTER IN SYMPATHETIC GANGLIA OF THE FROG. Yuh Nung Jan*, Lily Yeh Jan* and Stephen W. Kuffler. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

A peptide that resembles luteinizing hormone releasing hormone (LHRH) was found to be a likely transmitter for the late slow excitatory postsynaptic potential (epps) that lasts for many minutes in the bullfrog sympathetic ganglia, because (1) Radioimmunoassays established that 100-800 pg of a LHRH-like substance is contained in the lumbar chain of sympathetic ganglia. The peptide is confined to those spinal nerves that contain a distinct group of preganglionic axons which initiate the late slow epps, (2) Five days after ipsilateral preganglionic axons are cut, 95% of the LHRH-like material disappears from ganglia, while the LHRH immunoreactivity triples in the spinal nerves proximal to the cut region, (3) The LHRH-like substance is released by raising the external potassium concentration; this release is calcium dependent. (Proc. Natl. Acad. Sci. USA 76 1501-1505, 1979).

Recent electrophysiological experiments have shown that LHRH or two of its analogs, when applied by pressure from a micropipette to the cell surface, produce a depolarization of long latency and slow time course similar to that of the late slow epps. The conductance changes during the peptide-induced depolarization are also comparable to those during the late slow epps. Further, a potent antagonist of LHRH in mammalian systems blocks the nerve evoked late slow epps as well as the LHRH-induced depolarization, suggesting that the late slow epps is mediated by a LHRH-like peptide.
SITE OF ENKEPHALIN ACTION IN THE HIPPOCAMPUS. Robert A. Jensen, Joe L. Martinez, Jr., Robert Greaser, John Veltiugine, James L. McNaughton, and Lynn Chisholm. Department of Psychology, School of Biological Science, Indiana University, Bloomington, IN 47401.

Evidence indicates that enkephalins exert a powerful modulating influence in the operation of the hippocampus. We now report that enkephalins exert a similar influence on the integrity of hippocampal neurons. In these studies, the enkephalin analogs D-Ala-Leu-enkephalin (H-Lys-Tyr-D-Ala-Gly-Phe-D-Leu-OH) or naloxone was superfused over the tissue in the absence and presence of colchicine and the electrical response to stimulation was recorded. Hippocampal slices (600 µm) were placed in a recording chamber and maintained in a Krebs-Ringer bicarbonate medium (3% CO2/97% O2) at 33° C. Bipolar stimulating electrodes were placed in the Schaffer-commissural projections extending to the pyramidal cell dendrites. The action potentials were also placed in the alveus, the fiber bundle that carries the axons of the pyramidal cells. These two electrode placements allowed for both orthodromic and antidromic activation of the pyramidal cells. One recording microprobe was placed in the region superior to the pyramidal cell body layer to record the "population spike" response of these cells to stimulation.

The "population spike" response of pyramidal cells to Schaffer-commissural stimulation was highly sensitive to the enkephalins and demonstrated dramatic increases in amplitude even with concentrations as low as 10 nanomolar. The onset of this effect was rapid and was reversed by perfusion with peptide-free medium. Naloxone, by itself, had no detectable effect on this response, but it blocked the effect of enkephalin. Additionally, enkephalins in concentrations in the micromolar range, enkephalin had no observable effect on synaptic potentials generated by Schaffer-commissural stimulation nor did it affect the action potential recorded in the cell body layer to stimulation of the alveus.

Taken together, these findings suggest that enkephalin acts to attenuate the phase 2 inhibition of the population spike and is effective at concentrations in the low micromolar range. These observations are consistent with those that have been reported for the rat neocortex (Hajos, 1980; Hajos and Sheard, 1978) and the midbrain raphe (Dichter et al., 1980) and confirmed the presence of enkephalin-rich brain regions, but interpretation of this effect is unclear. According to Belluzzi et al (1977), who assume that positive reinforcement is mediated in part at opiate receptors, naloxone blocks enkephalins with at least the same extent as those that did display analgesia (group + -). Since naloxone (group + -) were suppressed by naloxone to at least the same extent as those that did display analgesia (group + +). Surprisingly, self-stimulators that failed to exhibit analgesia (group + +) were positive for SS; these were evaluated for sensitivity of naloxone suppresses SS merely by increasing aversion was not supported. Rather, the data are consistent with the hypothesis that naloxone blocks endorphin-mediated reward.
1789 METHIONINE-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN FETAL RAT BRAIN CELLS IN AGGRégATING CULTURE AND IN MOUSE NEUROBLASTOMA CELLS. Elinor L. Knodel* and Elliott Richelson. Depts. of Psychiatry and Pharmacology, Hayo Fdn., Rochester, MN 55901.

Brains from fetal rats of 14-16 days gestation were mechanically dissociated and grown as aggregates in rotation culture. The aggregates were fixed in buffered 4% paraformaldehyde and 8 μm frozen sections were prepared. Sections were stained by the indirect immunofluorescence method using antibody to methionine-enkephalin (a gift from R. Elde). The specificity of the reaction was determined by staining with antiserum pre-incubated with 1 μg/ml methionine-enkephalin.

In aggregates cultured for 2 or 3 days, only background fluorescence was observed; however, by 6 days many aggregates contained specifically-labeled cells, which were generally scattered throughout the aggregate. Occasionally, fluorescent cells were clustered in groups in the central portion of an aggregate, known to be comprised mainly of neuronal cells in older aggregates. After 4-5 weeks in culture very few aggregates had brightly fluorescing cells in their central areas. Both cell bodies, excluding nuclei, and cell processes were labeled. Incubation of the aggregates overnight with 10^-5 M morphine had no effect on the fluorescence.

About 5% of mouse neuroblastoma cells (clone NIE-115) in stationary phase also contained methionine-enkephalin-like immunoreactivity, but the staining pattern differed from that seen in the fetal brain cells. Occasionally whole cells were labeled, including their nuclei, of the culture-staining neuroblastoma cells contained one or two brightly labeled inclusions, much larger in size than the storage vesicles for neurotransmitters. Thus, although these cultures of neuroblastoma and fetal rat brain cells contain few specific binding sites for opiates, the present results suggest that they may synthesize and store endogenous opiate-like peptides.

(Supported by Mayo Foundation and USPHS Grants DA 1490 and MH 27692.)


Recent studies have shown that ACTH has direct neurotrophic activity. This study was undertaken to determine whether ACTH treatment can accelerate the rate of regeneration of crushed peripheral nerve and denervated muscle in intact and adenolyzed (adx) rats. Animals were treated with 0.25 U of ACTH 1-39 daily following nerve crushing for periods of up to 40 days. The regeneration rate was measured by foot-flick response to pain, distance between 1st and 5th digit (dl-5), average length of regenerated axons, and incorporation rate of amino acids by the denervated muscles.

ActH-treated adx rats showed increased sensation and functional movements more rapidly than did untreated groups. Dl-5 and foot-flick response to pain showed similar results. The average length of regenerated axons nine days after crushing for the adx-ACTH group was significantly longer (3.99 ± 0.47 mm) than that of adx-saline group (2.68 ± 0.36 mm). The incorporation rate of amino acids in adx-ACTH group, as measured 9 days after denervation, was increased significantly over that of untreated adx controls. Histological and histochemical examination of the muscles showed that ACTH has no effect on muscle weight, fiber diameter and fiber types as compared to their control groups. However, increase in number of enlarged endplates and more endplates with branched pre-terminal fibers were observed.

1792 NEUROTOXIC AGENTS REDUCE SOMATOSTATIN CONTENT OF THE RAB NEURAL RETINA. Nora Lake and Yoseph G. Patel*. Dept. of Research in Anesthesia, and Fraser Labs, Dept. of Medicine, McGill University, Montreal, Quebec, Canada, H3G 1Y6.

Recent reports by ourselves and others have described the presence of somatostatin-like immunoreactive material (SLI) in the retina of various mammals. In an attempt to utilise this we have utilised neurotoxic agents and have examined their effects on retinal SLI and morphology. Kainic acid is a glutamate analog which exerted its effects on CNS neurones and, when given intracranially to young chicks, causes marked degeneration of cells in the inner nuclear layer of the retina, particularly amacrine interneurones (Lodewycz and Cople, 1977, Invest. Ophth. Vis. Sci. 16, 401). We have found similar degeneration in the retina of adult rats injected intracranially 48 hours previously with 120 nmol of neutralized kainic acid. This was associated with a marked reduction of retinal SLI as measured by radio-immunoassay, from 1.24 ± 0.14 pg SLI/mg protein in saline controls (n=7) to 0.38±0.05 pg SLI/mg protein in kainic acid treated rats (n=6). The second retinotox compound which we examined was monosodium glutamate (MSG) which we administered subcutaneously (2 mg/g body weight) on a daily basis to rats for the first ten days of postnatal life. Histological examination of the retinas from 23-25 day old MSG-treated rats showed considerable degeneration in the inner plexiform layer and cell loss in the inner nuclear layer of ganglion cells. Concurrently there was a severe reduction of retinal SLI to 10% or less of control levels. These studies suggest the localization of retinal SLI within amacrine interneurones of the inner retina, perhaps within some amacrine and ganglion cells. SLI is present in sufficient quantities to suggest that it may have a neurotransmitter or neuromodulator role at these sites in the neural retina.
NEUROPEPTIDES

1793 IS THERE A ROLE FOR THE ENDOGENOUS OPIOID PEPTIDES IN THE REGULATION OF BLOOD GLUCOSE? Claudia Landau*, Roberta Palmour*, and J.-E. Cheng*. (SPON: L. Mathers). Department of Physiology, Faculty of Medicine, Univ. of Man., Winnipeg, Canada, R3E 0W3.

The presence, distribution and pharmacological properties of highly specific opioid receptors within the central nervous system and in anterior pituitary have been the focus of intensive research. Several lines of evidence suggest that opioids and opiate receptors exist in other peripheral tissues. Preliminary evidence indicates that specific opioid binding sites in rat kidney and adrenal glands. While many of the reported effects of opioid peptides may be mediated through central or peripheral receptors, changes in the level of opioid peptides may also play a role in physiological regulation. Holaday et al. (PNAS 74:6582, 1977) found that adrenalectomized (Adx) mice responded to iv β-endorphin with severe respiratory depression, cyanosis and seizures, while intact mice did not. The suppression of these effects by pretreatment with desmethyloexene, the similarity between these toxic effects and insulin shock, and the recent report of Ipp et al. (Nature 276:190, 1978) that β-endorphin stimulates insulin release in vitro, suggests that a relationship between glucose regulation and opioid peptides may exist.

We have tested the possibility that opioid peptides participate in the control of glucose regulation in intact and adx rats. Saline, insulin, morphine, β-endorphin and the enkephalin analog Tyr-D-Ala-Gly-N-Me-Pha-Met(0)-ol (PH625) were administered iv to intact and intact rats. Blood was drawn at 0, 15, 30 and 60 min. glucose, insulin, glucagon and endorphin levels were measured.

Core body temperature, respiratory rate, blood pressure and other visible autonomic signs were monitored. Gross behavior and catalepsy were evaluated.

Adx rats given insulin iv exhibited signs of severe respiratory depression, peripheral cyanosis, hypothermia and fall in core temperature. Adx rats given morphine showed typical opioid effects—muscular rigidity, cataleptic immobility and peculiar posture. Adx rats treated with 2.5 µM of PH625 showed autonomic effects similar to, but more severe than those seen in insulin-treated animals. Following ip injection of 10 mg glucose, respiratory depression, hypothermia and hypoglycemia were reversed, unmasking typical opioid effects.

Comparison of the opioid and glucoregulatory effects of β-endorphin and PH625 will be discussed as well as general physiological effects of these peptides.

1794 EFFECTS OF SOMATOSTATIN ON THE ATPase ACTIVITIES OF SYMPATOSOMES OF RAT CEREBRAL CORTEX. Sheu L. Lee* and Viktor Havlicek. Dept. of Physiology, Faculty of Medicine, Univ. of Nan., Winnipeg, Canada, R3E 0W3.

Somatostatin has been found to be present in many structures of the rat brain, including cerebral cortex. Sympatomatic pell of the cerebral cortex has 40 times higher concentration of somatostatin than the whole cortex. Upon cell depolarization with high extracellular potassium ions, stimulation of the cerebral cortex show calcium dependent release of somatostatin. All these findings allow us to speculate that somatostatin is a putative neurotransmitter or neurohormone in the cerebral cortex (Havlicek and Friesen in Central Nervous System Effects of Hypothalamic Hormones and other Peptides, Ed. R. Collu, A. Barbeau et al., Raven Press, N.Y., 1979, 361-402). In the present study we have found that somatostatin inhibits the in vivo ATPase activity of Sympatosome fractions (EC 3.6.1.3) in rat cerebral cortical sympatosomal fraction. The activity of Mg-ATPase was not significantly affected (Fig. 1) and there was no detectable effect on Ca-Mg-ATPase (for methods see Lee and Phillips, Can. J. Physiol. Pharmacol. 55, 961-969, 1977). Decrease of the ouabain-sensitive Na–K–ATPase of the sympatosomal membranes by somatostatin will result in depolarization of the membrane potential, which is in accord with results of in vivo experiments in which somatostatin applied iontophoretically stimulates activity of cortical ATPase (Miron, Ann. N.Y. Acad. Sci. 292: 433-438, 1977) and caused membrane depolarization (see Havlicek and Friesen, 1979).


The giant dopamine neuron (GDN) in the neonatal rat is one of the few central dopaminergic neurons in which the synthesis and release of an endogenous neurotransmitter or neurohormone has been investigated in detail. The dopaminergic neuron is a large cell with a long axon that arises in the substantia nigra and terminates in the neostriatum (Volle et al., Brain Research, in press). In the present study, we studied the effects of β-endorphin and somatostatin on the electrophysiological properties of these neurons in vitro.

In vivo experiments in which somatostatin applied iontophoretically to the neostriatum will result in depolarization of the cell membrane (Lichtensieger et al., 1976). In this study, we have observed that β-endorphin and somatostatin will also depolarize these neurons. Preliminary results from this laboratory describe specific sites on the cell membrane of these neurons that are sensitive to β-endorphin and somatostatin, and that these effects are blocked by the specific antagonist, SR48967.

In conclusion, these results indicate that in those conditions where plasma and cerebral fluid melatonin would be expected to be decreased, pituitary levels of β-endorphin would be significantly elevated. Melatonin administration, on the other hand, produced a significant decrease in pituitary β-endorphin. These studies suggest a complex interaction between pineal melatonin and pituitary β-endorphin, and further implicate the pineal gland in the physiological regulation of pituitary peptide hormones.


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α-L-ASPARTYL-L-ALANINE INCREASES MEPP AMPLITUDE. Ramon Lim, Shu Yin Cheung* and John W. Crayton, Brain Research Institute and Dept. of Surgery (Neurosurgery) and Psychiatry, University of Chicago, Chicago, Ill. 60637

α-L-Aspartyl-L-alanine (asp-ala) is a dipeptide we isolated from the pig brain (Cheung & Lim, Biochim. Biophys. Acta, in press). The biological activity of asp-ala is tested on a rat phrenic nerve-diaphragm preparation. At 10^−4 M asp-ala enhances neuromuscular transmission by increasing the amplitude of MEPP by 35%. The increase is noticeable during the first 30 min. of intracellular recording and is sustained up to at least 120 min. in the presence of the drug (see table and tracings below). However, the effect is maintained even after washing out the drug following the first 30 min. The free amino acids L-aspartic acid and L-alanine do not show noticeable effects under similar conditions. Another dipeptide, α-L-aspartyl-glycine, which has been isolated from urine but not from the brain, also does not have any effect. The results suggest asp-ala as one of the neural peptides capable of modulating synaptic transmission. (Supported by USPHS grant No. HS-09228)

**Time course of asp-ala effect on rat diaphragm**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No. of Fibers</th>
<th>Amplitude of MEPP (mV)</th>
<th>Frequency of MEPP (No. per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-drug</td>
<td>9</td>
<td>0.59 ± 0.02 (N.S.)</td>
<td>122 ± 39 (N.S.)</td>
</tr>
<tr>
<td>0-30'</td>
<td>9</td>
<td>0.80 ± 0.12 (p &lt; .002)</td>
<td>108 ± 45 (R.S.)</td>
</tr>
<tr>
<td>30'-60'</td>
<td>9</td>
<td>0.76 ± 0.12 (p &lt; .002)</td>
<td>125 ± 39 (R.S.)</td>
</tr>
<tr>
<td>60'-90'</td>
<td>9</td>
<td>0.78 ± 0.12 (p &lt; .002)</td>
<td>112 ± 31 (R.S.)</td>
</tr>
<tr>
<td>90'-120'</td>
<td>9</td>
<td>0.77 ± 0.12 (p &lt; .002)</td>
<td>124 ± 44 (R.S.)</td>
</tr>
</tbody>
</table>

(Each tracing is a composite of 20 sweeps)

CHARACTERIZATION OF MET- AND LEU-ENKEPHALIN RELEASE FROM RAT STRIATUM: INHIBITION BY ACETYLCHOLINE. Iris Lindberg* and June L. Dahl. Dept. Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis. 53706

The release of met- and leu-enkephalin from superfused striatal slices was studied. Pooled caudal striatal sections from five rats were chopped into two 0.3 mm slices and divided between four superfusion filters. The slices were superfused for 15 min. with warm oxygenated Krebs-bicarbonate (containing 60 µg/ml bacitracin) at a rate of 0.35 ml/min. Endogenous release was measured at 15 min. intervals. After the collection of three baseline fractions, stimulated release was elicited by a 4-min. exposure to Krebs containing 50 µM acetylcholine, with washout of the Krebs. Enkephalins in slices and perfusates were measured by radioimmunoassay (leu-enkephalin antibody was a gift of Dr. Richard Miller; met-enkephalin antibody was purchased from Immunonuclear Corp.). Results are expressed as the percentage of the initial tissue enkephalin released per min.

The mean basal release rates ± SEM of met- and leu-enkephalin were 0.21 ± 0.028/min. and 0.50 ± 0.076/min., respectively. After subtraction of basal release rates, the net increase in leu-enkephalin release induced by exposure to 50 µM potassium was 1.36 ± 0.164/min., whereas that of met-enkephalin was 1.22 ± 0.152/min. No potassium-stimulated increase could be observed in the absence of calcium. When the calcium concentration was increased to 2.5 mM, the basal release rates of leu- and met-enkephalin were inhibited by 55% and 99%, respectively; net potassium-stimulated release was inhibited 70% (leu-enk) and 18% (met-enk). 100 µM acetylcholine virtually abolished potassium-stimulated release, reducing basal and stimulated release to 55%. At the higher concentration of acetylcholine, basal release of met-enkephalin was inhibited by 44%, while stimulated release was inhibited by 62%.

While a greater proportion of tissue leu- than met-enkephalin was released, the absolute amount of met-enkephalin released exceeded that of leu-enkephalin. The release of met- to leu-enkephalin was 1.25 ± 0.10 in baseline fractions but rose to 2.23 ± 0.12 during potassium stimulation. The latter value more closely approximates the ratio of the enkephalins found after perfusion (3.35 ± 0.10). It is likely that the lower ratio found in the superfusates is related to the differential degradation of the two peptides (Bayou et al. 1983, J. Neurochem. 39, 75, 3503); this possibility is currently under investigation. (Supported by DA 00697; Iris Lindberg is an NIH Predoctoral Trainee supported by GM 10705.)


In this study, α-MSH (or α-MSH-like immunoreactivity) has also been detected in cat brain. Five mongrel cats of mixed gender were anesthetized with ketamine-xylazine and decapitated. The brains were removed and frozen on dry ice. The brains were alternately sliced into 200 µm thick sections by a Vibratome. Sections (100 µm) were reacted with a rabbit antiserum raised against the synthetic 1-25 α-MSH coupled to keyhole limpet hemocyanin. After washing, the sections were reacted with peroxidase-antiperoxidase (PAP) complexes. Paraformaldehyde-fixed monolayers of neuronal cultures grown in dissociated cell culture were examined by immunocytochemistry for endogenous neuronal peptides. Neurons of the cat brain and neuronal processes in the rat brain. These data indicate that α-MSH is differentially distributed within different nuclei of the cat brain and lend support to the hypothesis that α-MSH may be a CNS neurotransmitter or neuroregulator.
1.803 SOMATOSTATIN AND SUBSTANCE P LEVELS IN CULTURED SENSORY NEURONS

PepTeDi MODULATION OF ADRENAL PARANEURONS. Funio MiSeb*, Deanne M. Deau*, and Bruce G. Livett, Division of Neurology, The Neurological General Hospital and McGill University, Montreal, Canada

Adrenal paraneurons provide a model tissue culture system of neuroepithelial cells of neural crest origin. It has been clearly shown that adrenal medullary chromaffin cells can be isolated in a highly purified form by retrograde perfusion with collagenase and complete ganglionic separation on Percoll gradients1,2. When plated on collagen-coated plastic tissue culture dishes under conditions known to favor sympathetic neurite outgrowth in culture, these adrenal chromaffin cells produce long catecholamine-containing varicose processes and exhibit a Ca2+-dependent release of $^{3}H$-NE characteristic of noradrenergic neurons in culture3. Pharmacological studies have shown that the cells have nicotinic not muscarinic receptors4. The availability of this homogeneous adrenergic cell system has enabled a study to be made of the role of peptides as neuromodulators.

Substance P (10-8–5×10-7M; ID50 = 1.3×10-6M) and somatostatin (ID50 = 1.8×10-6M) produced a dose-dependent inhibition of the ACh (5×10-5M) and nicotine (5×10-6M) stimulated 3H-NE release from these adrenal paraneurons. In contrast, the K+ (56mM) induced release (5× 6mM K+) was not inhibited, and neither SF or somatostatin (10-6–5×10-5M) had any effect on the release of 3H-NE in the absence of the nicotinic agonists. Other peptides known to be present in the adrenal medulla, beta-endorphin, leu-enkephalin and met-enkephalin inhibited the ACh (5×10-5M) but not the K+ (56mM) induced release only at peptide concentrations greater than 10-7M (ID50 approximately 3×10-6M). Whether this pharmacological observation of the role of the cyclic opioid peptides is physiologically significant is presently under investigation using peptide inhibitors.

The results indicate a role for these peptides as putative neuromodulators in the nervous system.

(Supported by MRC and NIMH)

1.804 NEUROTIC ACTION OF CAPSAICIN ON SPINAL SUBSTANCE P NEURONS.

BOMBESIN-LIKE PEPTIDES: NEUROTTRANSMITERS IN MAMMALIAN BRAIN?


Bombesin, a tetradecapeptide isolated from frog skin, is active in the gastrointestinal tract and brain. This fact suggests that the nervous system bombesin induces hyperglycemia and hypothermia with a well-defined structure activity relationship which corresponds to that required for receptor labelling. Subsequently, bombesin-like immunoreactivity was detected in discrete varicose nerve fibers in a high regions (nucleus tractus solitarius, interpeduncular nucleus, median eminence, arcuate nucleus and substantia gelatinosa) than low regions (caudate, hippocampus and cingulate cortex). After intraventricular injection of vinblastin, neuronal cell bodies were observed in the spinal cord, hynpothermia and mamyillary nuclei as well as the mesencephalum. The distribution of bombesin-like peptides and substance P is strikingly coincidental, however, radioimmunoassay analysis of high-pressure liquid chromatographic fractions of brain extracts indicates that the two peptides are distinct.

Also, the subcellular distribution of bombesin receptors and bombesin-like peptide binding sites was investigated using our radioreceptor assay and radioimmunoassay. The greatest number of bombesin receptors and bombesin-like peptides is present in the synaptosomal fraction, suggesting an association with nerve terminals.

In addition, the release of bombesin-like peptides from rat hypothalamic slices was investigated. The amount released increased 2-3 fold when the brain slices were treated with depolarizing stimuli such as K+ or veratridine in the presence of Ca++. These results suggest that endogenous bombesin-like peptide may have a physiologic role in the synaptic function, either as neurotransmitters or neuromodulators.

The present values are consistent with the proposal that at least some primary sensory afferent neurons may utilize substance P as a neurotransmitter of nociception. Also supported by our findings is the emerging view of an intimate association between substance P and enkephalin neurons in pain mechanisms in the dorsal horn. If continued investigation shows the neurotoxicity of capsaicin in the neonate to be highly selective for substance P neurons of the spinal ganglia, then it should prove to be a useful and important tool for the determination of the precise mechanisms by which substance P neurons participate in the transmission of specific sensory modalities.

(These experiments were carried out in the laboratories of G.D. Fishbach and S.E. Leeman, and were supported by NIH grants GM 09919 and AM 16510.)

Neurotensin (NT), an endogenous tridecapptide distributed heterogeneously in the central nervous system of a variety of mammalian species, has previously been demonstrated to produce diminished locomotor activity, hyperthermia, potentiation of barbiturate-induced sedation and antinociception after intracisternal (IC) administration in mice and rats. Because of similarities between certain of the properties of acknowledged neuroleptic agents and NT, evaluation of the effects of the peptide in a variety of pharmacological screening procedures was performed. The administration of the endogenous tripeptide triptorelin-releasing hormone (TRH) abolished this effect of both neuroleptic agents and NT. The administration of NT for behavioral effects (locomotor activity and rearing) induced by d-amphetamine (2 mg/kg IP). Further studies sought whether IC NG in mice blocks the behavioral effects of peripherally administered apomorphine in a paradigm designed to test striatal (and mesolimbic) DA receptors (Psychopharmacol 50:1-6, 1976). NT showed no activity in this test. In the final series of experiments NT-induced antinociception was assessed and compared with the activity of ten other endogenous peptides. Adult male mice received IC ICNT injection of 1 µg NT (or another peptide in an equimolar dose) or vehicle and tested for antinociceptive activity by the Janssen method (measurement of latency to withdraw tail from 45°C water bath; Arzneimittel-Forsch 13:502-507, 1963). (β-enkephalin was the most potent antinociceptive agent tested but NT was quite potent, possessing significantly greater activity than met-enkephalin, leu-enkephalin and β-endorphin). Conclusion: NT possesses significant antinociceptive activity after IC administration. (Supported by NIMH MH32939, MH32316, and NICHD HD03110)


HPLC provides a rapid means for resolving and quantitating mixtures of closely related peptides. For example, peptides difficult to separate using one technique (e.g., an amino acid analyzer) may be more easily resolved by reverse phase HPLC. The present authors used HPLC for the separation of neuroactive peptides from crude tissues extracts. Using a solvent system composed of acetonitrile and triethylamine, peptides were separated and identified by amino acid analysis of fractions collected from the column.

1807 SOMATOSTATIN EFFECTS ON GLUTAMATE AND GABA TRANSPORT IN FROG SPINAL CORD. A.L. Padjen* & R.A. Davidoff, Depts. of Pharmacol. & Ther., McGill Univ., Montreal, Quebec H3C 1V6 and Neurology Service V.A. Hospital & Dept. of Neurology, Univ. of Miami, School of Medicine, Miami, FL 33132.

Somatostatin is present in frog spinal cord, presumably in primary afferents (unpublished observations), as in mammals. However, effects of somatostatin on synaptic transmission do not favor its role as a primary afferent excitatory transmitter but suggest a possible "neuromodulatory" role (cf. Padjen, Neurosci. Abs. 3, 411, 1977). In concentrations of 10−6-10−7 M somatostatin caused selective dose dependent depression of glutamate but not of GABA evoked responses on both homoneurons and primary afferents recorded extracellularly and by viral injection of radiolabeled faciliated uptake into fused frog spinal cord (somatic transmission blocked by TTX or high Mg). These experiments were extended to an analysis of the effects of somatostatin on the uptake and release of 3H-glutamate and 3H-GABA in frog spinal cord slices (for methods see Davidoff & Adair, J. Neurochem. 24, 454, 1975; Brain Res. 118, 403, 1976). Somatostatin (10−7 M) caused a small but significant increase in (3H)glutamate (10−7 M) high affinity uptake (+ 20%; control: 0.277 ± 0.031 SEM umoles/mg/min with somatostatin: 0.333 ± 0.045; n = 6) but no change in (3H) GABA (10−7 M) high affinity uptake (+ 56%; control: 0.386 ± 0.063; with somatostatin: 0.404 ± 0.061; n = 6). On the other hand the increase in (3H)glutamate release evoked by 40 nM K + was significantly depressed by the presence of 10−7 M somatostatin: +1565 ± 7.0 of resting efflux; with somatostatin: +114.35 ± 6.1; n = 6) without affecting K + evoked release of (3H) GABA (no somatostatin: +15.7 ± 12.6; with somatostatin: +158.3 ± 7.8; n = 6). These results suggest that somatostatin specifically interferes with glutamate transport (at high Mg). They contrast with somatostatin produced facilitation of ventral root potentials evoked by dorsal root stimulation, a pathway which is presumed to be mediated in part by glutamate.

(Supported by MRC of Canada).
CATECHOLAMINERGIC MODULATION OF ENKEPHALIN ACTION. M.R. Palmer, J. Stewart, M. Perlow, W. Freed, R. Wyatt, B.J. Hofer. Deps. of Pharmacology and Biochemistry, University of Colorado Medical Center, Denver, Colorado, and NIMH, St. Elizabeth’s Hospital, Washington, D.C., 20032.

The interaction between iontophoretically applied D-Ala² methionine enkephalinide and dopamine receptors on frontal cortex was investigated in rats. The depression caused by iontophoretically applied D-Ala² was similar to those observed after microinjection of the enkephalide, thus showing that the depression are not artificats of the iontophoretic technique. Antipsychotic agents known to block catecholamine receptors such as spiperone, α-fluphenthixol and (-) butaclamol, reversibly antagonized the depression caused when administered i.v. The biochemically inactive isomers of these antipsychotics, spiroperidol and (-) butaclamol, had no such effects. Unilateral lesions of the catecholaminergic projections to frontal cortex produced by interstitial injection of 6OHDA resulted in an ipsilateral decrease in 2dA efficacy, and in an elimination of the antagonism of 2dA depressions by antipsychotic agents.

1814 ELECTROPHYSIOLOGICAL RESPONSES TO ANGIOTENSIN II AND LUTEINIZING HORMONE. B. E. Hearst and Hereditary Disease Foundations. N.W. Pedigo, T. D. Reisine, N. C. Linga, E. D. Birk, L. L. Iversenb and H. I. Yamamura, Dept. of Pharmacology, Univ. of Arizona, Tucson, AZ 85724, *Neuroemodocrinology Lab, The Scripps Clinic, La Jolla, California, and Department of Pharmacology, NEUROPEPTIDES

1811 ELECTROPHYSIOLOGICAL RESPONSES TO ANGIOTENSIN II AND LUTEINIZING HORMONE. M. Ian Phillips and Dominik Felix, Dept. Physiol. & Biophys., Univ. of Iowa, Iowa City, IA 52242 and Brain Res. Inst. Univ. of Zurich, Zurich, Switzerland.

Angiotensin II (AI) and luteinizing releasing hormone (LH-RH) are neuropeptides which both produce behavioral effects when injected into the brain. Interest in the brain circumventricular organs as possible receptor sites for central effects of AI have focused on the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT). Previously, we have shown that the SFO in cats contains cells which are responsive to AI when it is applied directly to the vessel. In the present study, we have used a ventral approach to the OVLT of the rat for direct application by microiontophoresis of AI. Rats were anesthetized with urethane and the site was revealed by dissection. Extracellular action potentials were recorded through the central barrel of a 5-barrel glass microcathode. This contained 4 M NaCl and fast green dye. The tip diameter was approximately 4µm with a resistance of 4-12MΩ. Pain reactions were observed and the enkephalinide was prepared as a 10⁷ M solution in distilled water with a final pH of 4.5. Saralasin (Beckman) was iontophoretically applied at 5.5 angiotensin II (Calbiochem.), prepared as a 10⁻³ M solution in 165 mM NaCl (pH 7.4) was ejected electroosmotically from seven-barreled microelectrodes onto dorsal horn neurons whose firing rates were decreased by Saralasin. Dose response curves to AI were established in several of these cells. Cells which responded to AI were non-synaptic in origin, as demonstrated by microinjection of H-S binding sites in rat frontal cortex, corpus striatum and nucleus accumbens-olfactory tubercle. The biochemically inactive isomers of these antipsychotics, spiroperidol and (-) butaclamol, had no such effects. Unilateral lesions of the catecholaminergic projections to frontal cortex produced by interstitial injection of 6OHDA resulted in an ipsilateral decrease in 2dA efficacy, and in an elimination of the antagonism of 2dA depressions by antipsychotic agents.

THE EFFECTS OF COMBINED ANGIOTENSIN II (AI) AND LUTEINIZING HORMONE (LH-RH) ON SPINAL CORD DORSAL HORN NEURONS. T.F. Podolsky and M.T. Smith, Dept. Pharmacology, University of Nebraska Medical Center. The interaction between iontophoretically applied D-Ala² methionine enkephalinide and dopamine receptors on frontal cortex was investigated in rats. The depression caused by iontophoretically applied D-Ala² was similar to those observed after microinjection of the enkephalide, thus showing that the depression are not artificats of the iontophoretic technique. Antipsychotic agents known to block catecholamine receptors such as spiperone, α-fluphenthixol and (-) butaclamol, reversibly antagonized the depression caused when administered i.v. The biochemically inactive isomers of these antipsychotics, spiroperidol and (-) butaclamol, had no such effects. Unilateral lesions of the catecholaminergic projections to frontal cortex produced by interstitial injection of 6OHDA resulted in an ipsilateral decrease in 2dA efficacy, and in an elimination of the antagonism of 2dA depressions by antipsychotic agents.

REFERENCES

1810 INVESTIGATION OF DES-TYROSINE³-⁴-ENDOPHIN ACTIVITY AT NEUROLEPTIC BINDING SITES IN RAT BRAIN AND OF OPIATE AND NEUROLEPTIC BINDING IN HUMAN SCHIZOPHRENIC BRAINS. M. F. Piercey, F. J. Eipshahr and L. A. Schroeder. The Upjohn Company, CNS Research, Kalamazoo, MI 49001.

Several substances P (SP) partial fragments were tested for their effects on caudal dorsal horn neurons and guinea pig ileum. Ileal segments were mounted in 5 ml baths of aerated Tyrode's solution (37°C). Potencies were measured as the doses required to cause half maximal contractions. C-terminal fragments as small as the hexapeptide (SP6) retained full SP potency. Potency of the C-terminal (SP8 and SP) and N-terminal (SP10, 20 and 30) fragments were much lower, while the C-terminal tripeptide (SP3) and the N-terminal decapeptide (SP100) were inactive. SP tachyphylaxis completely eliminated the effects of SP and its analogs, but not those of non-SP receptor stimulants (acetylcholine, bradykinin, serotonin). Thus all SP fragments elicited their contractions via SP receptor stimulation. Microiontophoresis was used to test the effects of the SP analogs on spinal cord dorsal horn neurons. Unanesthetized decerebrate cats with LI spinal sections were used. Analogs (1 mM in 165 mM NaCl) were ejected electroosmotically from seven-barreled microelectrodes onto dorsal horn neurons whose firing rates were monitored by a central recording barrel. Nearly all SP-excitatory cells were excited by C-terminal fragments as small as SP6; smaller C-terminal fragments only occasionally excited these cells. Surprisingly, the N-terminal analog, SP100, weakly excited about half of the SP-sensitive cells on which it was tested. The excitatory effects of SP fragments were slow, resembling that for SP. Thus, it is unlikely that these effects were due to its being broken down into a smaller, more rapidly acting fragment. None of the SP fragments excited SP-insensitive cells. Thus, it is concluded that the excitatory effects of all SP partial fragments were mediated through a common SP receptor located on specific dorsal horn neurons. Because SP6 was the smallest SP fragment possessing full biological activity of the intact molecule and the spinal cord, the SP receptors for both tissues must be quite similar. Since SP100 was weakly active in the dorsal horn, but not the ileum, these receptors cannot be totally identical.
THE ACTIONS OF THE MOLLUSCAN NEUROPEPTIDE FMRFamide ON A MOLLUSCAN NEUROMUSCULAR JUNCTION. David A. Price, Dep. of Biological Sciences, Florida State University, Tallahassee, FL 32306.

The molluscan neuropeptide FMRFamide (phenylalanine- methyl-arginyl-arginyl-trypalaen) has excitatory actions on the isolated radula protractor muscle of Busycicon contrarium. To determine what role, if any, this peptide might play in this function of this muscle, I examined the pharmacological interaction of FMRFamide with the presumed neurotransmitters, acetylated choline (ACH) and gamma-aminobutyric acid (GABA), compared them to its effects on contractions elicited by nerve stimulation.

Ach causes contractions of the radula protractor which can be relaxed with SHT, often with the simultaneous induction of spontaneous rhythmic activity. The main action of FMRFamide is ACh-like, though the FMRFamide induced contractions develop and decay more slowly than those induced by Ach. Since this action of FMRFamide is unaffected by Ach antagonists, it presumably, represents a direct action of the peptide on the muscle and not an action on Ach-containing nerves. However, low concentrations of FMRFamide (less than 10M) often cause a response resembling that seen with mixtures of Ach and SHT. Since this oscillatory response is somewhat sensitive to Ach antagonists and is easily desensitized, it might be mediated through the nerves.

If the myoepithlora in situ receiving the radula protractor is stimulated at low frequency (0.2/sec) with short (0.2 msec) pulses, a uniform train of distinct twitches is obtained. If these concentrations are less than 10M of FMRFamide in the bathing medium increase the twitch amplitude with no increase in the resting tone. Ach antagonists sharply decrease the twitch height. FMRFamide contraction amplitude diminishes in the absence of nerve stimulation relax when nerve stimulation is resumed. These results support the supposition that Ach and SHT are not the neurotransmitters in radula protractor neuromuscular junction. Though FMRFamide has direct actions on the radula protractor, it is apparently not a neurotransmitter. It might be acting as a neuromodulator.

ENTRY OF OPIOID PEPTIDES INTO THE CENTRAL NERVOUS SYSTEM.


Neuropeptides are a family of proteins that play a role in neurotransmission and are released into the systemic circulation. They can have actions similar to neuropeptides, though the main action of FMRFamide is ACh-like, though the FMRFamide induced contractions develop and decay more slowly than those induced by Ach. Since this action of FMRFamide is unaffected by Ach antagonists, it presumably, represents a direct action of the peptide on the muscle and not an action on Ach-containing nerves. However, low concentrations of FMRFamide (less than 10M) often cause a response resembling that seen with mixtures of Ach and SHT. Since this oscillatory response is somewhat sensitive to Ach antagonists and is easily desensitized, it might be mediated through the nerves.

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EFFECT OF CYCLO(LEU-GLY) ON MORPHINE DEPENDENCE AND MORPHINE-INDUCED DOPAMINE RECEPTOR SENSITIVITY

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The effect of cyclo(Leu-Gly) on the development of morphine tolerance and physical dependence as well as morphine-induced alterations in dopamine (DA) receptor sensitivity were assessed. Animals rendered physically dependent and tolerant to morphine by subcutaneous (s.c.) implantation of morphine (75 mg/kg free base) pellets for 3 days. A dose of 0.18 μg cycles of cyclo(Leu-Gly) injected i.p. 2 hours prior to the pellet implant prevented the development of physical dependence as measured by changes in body temperature observed during either abrupt or naloxone-induced withdrawal. Cyclo(Leu-Gly) also inhibited the development of tolerance to morphine analgesia; however, the acute treatment did not modify morphine analgesia in tolerant mice.

The dose of the DA agonist apomorphine (AP0) necessary to produce an increase in locomotor activity determined 24 hours after the removal of the pellets was in morphine-dependent mice relative to placebo controls. The pretreatment with cyclo(Leu-Gly) in the same manner which blocked the development of physical dependence and tolerance also prevented the increase in DA receptor sensitivity. Similar results were obtained with the hypothermic response to the DA agonist piretindol (20 mg/kg i.p.). Piretindol produced the hypothermic response (15 ± 3°C) in morphine-dependent mice given cyclo(Leu-Gly) than in dependent mice given saline injection. Mice pretreated with cyclo(Leu-Gly) and chronic morphine treatment did not differ from morphine-naive mice in their respective injection of cyclo(Leu-Gly) on the third day of morphine treatment, at a time when physical dependence and tolerance could be demonstrated, did not differ either the sign of physical dependence and tolerance, or DA receptor sensitivity. These data indicate that cyclo(Leu-Gly) administered prior to morphine administration in mice prevents the increase in DA receptor sensitivity which accompanies the development of physical dependence and tolerance to morphine. This work was supported by U.S. HS Grant AM-18399, by NSF Grant OB-42753 and BRS-73-1129, and by the Ill. Dept. Ment. Hlth. and Develop. Disabil. Grant 904-02.

NEUROPEPTIDES


There is growing evidence that the undecapeptide substance P (SP), among other peptides having CNS effects, may functionally interact with the neurochemical mechanisms of the basal ganglia, the site of the motor disturbances produced by substance P. Intraventricular injection of SP in a dose of 30 μg/rat increased motor activity in rats rendered hypokinetic by bilateral microinjections of 6-hydroxydopamine into the anterolateral hypothalamus. Behavioral observations indicated that grooming and motor activity were the most consistently and significantly expressed behaviors. The effects of the same doses of the peptide on the hypokinesia induced by α-methyl-para-toluidine, phenoxybenzamine, and haloperidol were then examined. SP did not affect the behavioral depression produced by α-methyl-para-toluidine (250μg/kg) and phenoxybenzamine (20 mg/kg). However, SP systematically reversed the decrease in locomotor activity induced by a relatively small dose of haloperidol (1.0 mg/kg). On the other hand, SP did not counteract the hypokinesia and other spastic symptoms resulting from the administration of a higher dose of this dopamine antagonist (3 mg/kg).

These results will be examined in light of the hypothesis that peptides may interact with endogenous trophic modulation on catecholaminergic functions and may offer a possible approach for the amelioration of the extrapyramidal disorders.

SP was supported by the Natural Sciences Council of Canada D.B.R. and F.B.J. are post-doctoral fellows from the NRC and CRQ respectively. G.F. is from Dept. Psych., Concordia Univ., Montreal, Quebec, Canada.

Bovine Adrenals Contain High Levels of Enkephalin and Even Higher Levels of Several Putative Enkephalin Precursors.

J. Boasler, R. Lewis*, A.D. Stern*, S. Stein*, and S.Udenfriend*.
Roche Institute of Molecular Biology, Nutley, NJ, 07110.

Adrenal medullae from several species have been shown to contain enkephalin-like materials by radioligand assays and by immunohistochemistry. Using HPLC and a fluorescamine detection system, we have now shown that bovine adrenals contain Met5- and Leu5-enkephalin and beta-endorphin. tryptic and chymotryptic hydrolyses of bovine adrenal medullae were homogenized in 1 N acetic acid, 20 mM HCl supplemented with 10 mM EDTA. Aliquots were analyzed for content by radioreceptor assay, two different enkephalin radioimmunoassays and amino acid composition. A typical preparation from 3 adrenals (6.5 gm) contained 1.57 nmol/gm Met5-enkephalin and 0.28 nmol/gm of Leu5-enkephalin. Similar values are found in the rat striatum. We also found that ER had significantly lower saccharin consumption than CR in Experiment 2; tended to drink less saccharin (trials 1-35) in Experiment 3.ER had access to tap water (Experiment 1) or were injected 90 min before access to .1% saccharin (sham conditioning) (Experiment 2 & 3). In each experiment (Experiment 3 within parentheses), recipients (ER & CR) were given 30 min access to .1% saccharin (12/24) hrs after the subdural (IP) injection. Recipients were given a lithium chloride injection IP 3 hrs after access to saccharin started. Twenty-eight hrs later and each subsequent day at the same time, recipients had simultaneous 6(1) hr access to .1% saccharin and tap water after taking licks from each solution. Analysis of variance for the group X trial interaction indicated that ER had significantly lower saccharin consumption than CR (trials 1-22 in Experiment 1 .p<.05) no difference in Experiment 2; tended to drink less saccharin (trials 1-35) in Experiment 3 (p<.1); and had highly significant lower saccharin consumption (trials 1-22 in Experiments 1 and 3 combined (p<.01) with no differences due to procedure disparities.

The independent and pooled data from Experiments 1 and 3 suggest that the brain extracts from ED fostered an enhanced aversion to .1% saccharin. In Experiments 1 and 2, rats were injected intraperitoneally (IP) with lithium chloride (aversive) after 30 min access to .1% saccharin on 2 consecutive days. Two hrs after the second condition- ing day, recipients were sacrificed by decapitation, brains homogenized, centrifuged and the supernatant lyophilized, all under cold conditions. Control donors (CD) underwent the same procedures except that they had access to tap water (Experiment 1) or were injected 90 min before access to .1% saccharin (sham conditioning) (Experiment 2 & 3). In each experiment (Experiment 3 within parentheses), recipients (ER & CR) were given 30 min access to .1% saccharin 12(24) hrs after the subdural (IP) injection. Recipients were given a lithium chloride injection IP 3 hrs after access to saccharin started. Twenty-eight hrs later and each subsequent day at the same time, recipients had simultaneous 6(1) hr access to .1% saccharin and tap water after taking licks from each solution. Analysis of variance for the group X trial interaction indicated that ER had significantly lower saccharin consumption than CR (trials 1-22 in Experiment 1 .p<.05) no difference in Experiment 2; tended to drink less saccharin (trials 1-35) in Experiment 3 (p<.1); and had highly significant lower saccharin consumption (trials 1-22 in Experiments 1 and 3 combined (p<.01) with no differences due to procedure disparities.

The independent and pooled data from Experiments 1 and 3 suggest that the brain extracts from ED fostered an enhanced aversion to .1% saccharin in ER. Since saccharin consumption of ER and CR are the same during the initial conditioning day, these results appear to be related to learning rather than some permanent change. In Experiment 2, a convulsive and lethargic reaction was observed after saccharin injection; this did not occur either Experiment 1 or 3. Results from Experiment 2 may have been confounded by this reaction.

The paradigm is novel as a model for behavioral-neurochemical investigations of the interanimal transfer of taste aversion (.1%, .5%, etc saccharin, maple, etc) and also those to purified peptides (SP, DR, FRH, etc).
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1821 PEPTIDE HORMONE EFFECTS IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. Ronald L. Seaman, Robert L. Moss and Martin J. Lynch*. Dept. of Physiology, University of Texas Health Science Center at Dallas 75235. †Present Address: Biomedical Research Group EHE/STL, Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia 30332. The abdominal ganglion of Aplysia californica was used to investigate the effects of peptides on the firing rates of invertebrate neurons. Abdominal ganglia from summer animals were studied in artificial seawater at 22 to 24°C. The peptides applied at 1 µM in artificial seawater. About one-half of the tested white-cell neurons (K3-R13) responded to luminal injection of hormone (Rao et al.). Responses to the bath application of this peptide were increased in firing rate which lasted for 20 to 80 minutes. The increased firing rate was returned to normal after the bath was returned to normal seawater. In one instance, a second application of LHRH applied at 80 minutes after an initial application produced a second increase in firing rate. The absence of a change in firing rate by the remaining white-cell neurons suggests that, in terms of LHRH sensitivity, there may be two types of white cells.

The activity of other neurons in the abdominal ganglion changed in the presence of peptides. Application of LHRH altered the firing pattern of burster L2 but did not affect activity in bursters L10 and R15. The firing of a darkly pigmented neuron near R15 slowed and stopped in the presence of Substance P (Bacheo). Activity did not resume for this cell when the bath was returned to normal seawater. Some neurons in the abdominal ganglion of Aplysia californica are sensitive to peptide compounds tested. Interneurons may be involved in some of the responses, but since the white cells have no synaptic inputs, the effects on them are probably direct. The effects, when observed, lasted for several minutes and continued after the peptide in solution had been removed. Both LHRH and Substance P were present in neuronal activity. Their actions on Aplysia neurons suggest these neurons as potential models for the mammalian nerve fibers of the effects of these peptides. The sensitivity of invertebrate neurons to mammalian hormones may be more common than originally thought. (Supported by a Biomed. Res. Support Grant through UTSCD to RLS, Project Program No. 09978 to RLM; and S.E. 007-04502-17.)

1822 PATHWAYS IN MOUSE AND RAT BRAIN CONTAINING Vasoactive Intestinal POLYPEPTIDE (VIP): AN IMMUNOCYTOCHEMICAL STUDY. Katherine B. Sime*, Donald L. Hoffman*, Karl A. Tatem*, and Samuel I. Sato†. †Department of Anatomy, Virginia Commonwealth University, Richmond, Virginia 23298. VIP is a 28 amino acid residue peptide originally isolated from gut, is known to be present in brain by bioassay, radioimmunoassay and immunocytochemistry. It is immunopositively distributed in cell bodies in cerebral cortex and amygdala and in nerve fibers innervating cerebral blood vessels and a number of nuclear regions including the paraventricular hypothalamic nucleus and parabrachial brainstem. Although reactive perikarya were reported in arcuate region of mouse hypothalamus, they were not found in rat. We have studied both mouse and rat brains by immunoperoxidase technique using peroxidase-antiperoxidase complexes (PAP) on 6 µm deparaffinized Bouin's immersion-fixed sections on glass slides and unmounted at 50 µm cryostat sections from animals perfused with paraformaldehydelysin-periodic acid and penetrated with Triton X-100. Preabsorption of anti-VIP with synthetic VIP abolished all reactivity while equimolar secretin, which shares structural homologies, had no effect. All previous immunocytochemical findings were confirmed except for the arcuate nucleus which was devoid of reactive cells. Instead, in both species, cell bodies containing VIP were found in the basal portions of the suprachiasmatic nucleus and their projections could be traced dorsally to the paraventricular, periventricular, and dorsomedial nuclei of the hypothalamus and on to the periventricular thalamus. In all other respects both species were identical as well. A much more extensive distribution of VIP was found than previously appreciated. Cell bodies were found to be scattered throughout the caudate, in dorsal parts of the substantia nigra, ventral to the aqueduct and extending ventrally in the midline, in ventrolateral medulla in the nucleus of the tract of the trigeminal and extensively into the substantia gelatinosa. A few cell bodies were also found in preoptic hypothalamus and bed nucleus of the stria terminals. Many fibers were found in these and other regions including the entire length of the spinal cord. The most dense projections were found in brain central nucleus of the amygdalas, and nucleus of the solitary tract in the medulla. It is apparent that VIP, like substance P and enkephalin, is widely distributed in brain and spinal cord in multiple pathways, and like other peptides thus far studied does not appear to innervate cerebellum.

1823 IMMUNEFFECTIVE THYROTROPIN RELEASING HORMONE (TRH) OUTSIDE THE HYPOTHALAMUS REALLY IS TRH. Eliot Spinlasi* and Richard Hurtman (SPON: N. J. Farnstrom). Massachusetts Institute of Technology, Cambridge, MA 02139. Controversy exists concerning the identity of immunoreactive TRH in brain areas outside the hypothalami. To establish its identity we analyzed regions of rat brain by thin layer chromatography (TLC), ion exchange chromatography, high pressure liquid chromatography (HPLC) and bioassay. By all methods of analysis, all TRH immunoreactivity corresponded exactly to that of synthetic TRH. TRH tissue was extracted with either 2N acetic acid or 90% methanol; then subjected to one of three different HPLC separations or two different TLC separations. Ion exchange chromatography on SP Sephadex was a convenient peptide extractive procedure. Their actions on Aplysia neurons suggest these neurons as potential models for the mammalian nerve fibers of the effects of these peptides. The sensitivity of invertebrate neurons to mammalian hormones may be more common than originally thought. (Supported by a Biomed. Res. Support Grant through UTSCD to RLS, Project Program No. 09978 to RLM; and S.E. 007-04502-17.)

1824 CORTICAL AND HIPPOCAMPAL SPREADING DEPRESSION INDUCED BY INJECTIONS OF METH- AND MENTHENKEPHALIN AND BY D-ALA2-METHENKEPHALINAMIDE. U. Sprick*, K. Orinstein*, M.-S. Olti* and J.P. Huston (SPON: A. Borbély). Institute of Psychology, University of Düsseldorf, 4000 Düsseldorf, FRG.

Leukenkephalin, metenkephalin and d-Ala2-met- enkephalinamide were microinjected into the hippocampus and neocortex of 250 rats to examine the effects on EEG and slow wave potentials. All substances were found to elicit spreading depression in both neocortex and hippocampus. A dosage required to elicit spreading depression in at least 50% of the trials was determined by testing doses of 100µg/µl, 50µg/µl, 12.5µg/µl, 6.25µg/µl and 3.125µg/µl as detected in µl of distilled water. The following minimal doses were required for the induction of SD in at least 50% of the trials:

Hippocampus Neocortex
Leukenkephalin 12.5 µg 6.25 µg
Metenkephalin 100.0 µg 100.0 µg
D-Ala2-Metenkephalinamide 12.5 µg 6.25 µg

Lower doses were required to induce hippocampal seizure activity in at least 50% of trials: leuken- kephalin - 12.5 µg, metenkephalin - 50.0 µg, and d-Ala2- metenkephalinamide - 50.0 µg. However, the prevention of the elicitation of spreading depression. The differences between the various enkephalins in terms of the extent of spreading depression could be related to different receptor or acceptor densities. It should be noted that some of the various report- ed behavioral effects of intracranial injections of the enkephalins could be artefacts of hippocampal and/or cortical spreading depression.
Inhibition of Evoked Unit Discharges in the Periaqueductal Gray by Hypothalamic Stimulation

Jean C. Strahlendorf, Howard K. Strahlendorf, and Charles D. Barnes. Department of Physiology, Texas Tech University School of Medicine, Lubbock, Texas 79430.

Available anatomical and immunohistochemical evidence supports the concept of an endorphin containing neuronal system which has its origin in the hypothalamic arcuate nucleus (Arc.N.), and projects via long axons to various brain structures including the periaqueductal gray (PAG). That the PAG is an important structure with regard to opiate analgesia derives from studies demonstrating a high density of opiate receptors within this area and numerous investigations showing the PAG to be analgesically active when tested with microinjected opiates. Beta-endorphin is particularly active in this sense, inducing intense prolonged analgesia when applied to the PAG. We have therefore investigated the effect Arc.N. activation on PAG activity.

Single cell activity was recorded with stainless steel microelectrodes stereotaxically directed at the ventral-lateral aspect of the mesencephalic PAG in chloralose anesthetized, paralyzed, and artificially ventilated cats. Evoked discharges were, however, induced by strong electrical shocks (> 2.0 mA) applied to the sural nerve exposed in the popliteal fossa. Brief trains (100 Hz, 3 pulses, < 500 µA) of conditioning stimuli were applied to the Arc.N. ipsilateral to the recording side. Four units encountered displayed spontaneous activity. Arc.N. stimulation produced a period of total cessation of cell firing lasting 250 msec in one instance but failed to affect the 3 remaining units. Eighteen cells which discharged in response to sural shocks were tested with Arc.N. stimulation (C-T paradigm). Arc.N. suppressed evoked discharges by an average of 54% (range 3-100%). Intravenously administered naloxone (Nal) 2 to 5 mg/kg failed to markedly attenuate Arc.N. elicited inhibition and did not affect sural evoked activity. These results indicate that the Arc.N. is capable of inhibiting evoked PAG single unit activity, but unlike effects observed in locus coeruleus (this meeting) this inhibition is not changed by the narcotic antagonist Nal.

Supported in part by NIH Grant HL7289 and the Tarbox Parkinson's Disease Institute of Texas Tech University School of Medicine.

Metabolism of the enkephaline in brain can occur via at least two different enzymatic routes. A soluble amineopeptidase can liberate the N-terminal tyrosine and a membrane-bound endopeptidase can cleave the gly-gly bond, thus liberating the tripeptide tyr-gly-gly (TGG) and phe–gly–gly (PGG). The endopeptidase (enkephaline) has somatotopic and regional brain distributions which correlate with the opiate receptor, whereas the aminopeptidase does not. Enkephaline is thought to be specific for the endogenous enkephalergic system. It is a metalloenzyme which is completely inhibited by 1,10-phenanthroline (LMM) and partially inhibited by EDDA (LMW). EDDA has little effect on enkephaline, thus indicating a lack of calcium dependence. The metal cofactor is apparently very strongly bound as an extensive dialysis does not affect enzymatic activity.

Enkephaline is inhibited by various enzyme resistant analogs of the enkephalins and by the peptides tyr-gly, phe–leu and gly-gly-d-phenylgly. Preliminary evidence suggests that enkephaline is inhibited by phosphorylating conditions. Further experiments are in progress to elucidate the mechanism of this inhibition.

BOMBESIN DISRUPTS THERMOREGULATION IN RATS AT HIGH AND LOW ENVIRONMENTAL TEMPERATURES. Yvette Taché*, Quentin J. Pittman and Harvin Brown (SPON: W. Yale). Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Bombesin, a tetradecapeptide originally isolated from frog skin causes hypothermia when given intracisternally to rats at low ambient temperatures (TA). The demonstration of bombesin-like immunoreactivity and bombesin-specific binding sites in rat brain raises the possibility that an endogenous bombesin-like peptide might play a role in thermoregulation in the rat. We have, therefore, undertaken the following studies to assess the action of bombesin on thermoregulation in unrestrained, unanesthetized rats.

Male Sprague-Dawley rats (250-300g) were implanted with 22 ga lateral ventricular cannulas. Following a minimum of 5 days recovery from surgery, rats were placed in individual cages in an environmental room where TA could be precisely regulated. Rectal temperatures (Tb) were recorded at 30 min intervals from 60 min to a minimum of 90 min after injection. Bombesin, or an inactive analog were injected in a volume of 10µl of sterile, pyrogen-free artificial CSF made up fresh prior to injection.

The effects of intraventricular (ivt) bombesin (1ng-10µg) on Tb were tested at TA's of 4°C, 24°C, 31°C, 33°C and 35°C. At all TA's tested, control injections of the bombesin analogs [D-Trp²]-bombesin or [D-Trp²]-bombesin were without effect on Tb. At 4°C, bombesin caused a reduction in Tb with a return to normal Tb within 3.5 hr following the threshold dose of 50ng. Higher doses caused a more profound and longer lasting hypothermia with Tb decreasing by a maximum of 7°C at doses of 500ng-1µg. At TA = 24°C, 1µg bombesin reduced Tb by 2°C, whereas administration of this amount at TA = 31°C and 33°C was without effect on Tb. However, at TA = 35.5°C, Tb was increased by 1.6°C (0.5–2.1°C, n = 11) with further increase apparent at a dose of 10µg bombesin. The thermoregulation observed at high Ta could be reversed to a hypothermia by transferring the rats to a TA of 4°C within 1 hr of drug administration.

These results confirm and extend previous findings by demonstrating a reversible hypothermic effect of bombesin administered ivt to the unanesthetized rat. Administration of bombesin to rats exposed to high Ta's revealed a hyperthermic action for this peptide. These findings demonstrate a disruptive effect of bombesin on thermoregulation at temperatures above and below thermoneutrality and suggest an inhibitory action on both heat production and heat loss pathways.


Plasma growth hormone levels fall and remain low for several hours after stress. This effect is partially reversed by iv administration of anti- somatostatin. The present study was undertaken to determine the role of CNS SHIF in stress-induced suppression of growth hormone secretion.

Adult male Sprague-Dawley rats kept on 14:10 light:dark cycle with food and water ad lib were forced to swim for 30 minutes beginning at 1000h in a tank filled with water at 37°C. The rats were sacrificed immediately afterwards by decapitation. Their brains were snap-frozen and serum collected from trunk blood. Serum rGH was assayed using NIH anti rat rGH. Somatostatin was determined in 10 microdissected, individual brain nuclei by a highly specific and sensitive radioimmunoassay.

Serum rGH was significantly reduced in stressed animals compared to "rested" controls (6.4±1.0 vs 49.5±12.5 ng/ml respectively). Swimming stress resulted in a significant reduction of SHIF in the median eminence (ME), the medial portion of the caudate nucleus (MCN) and the medial portion of the caudate nucleus (MCN) as measured by microdissection. Somatostatin was determined in 10 microdissected, individual brain nuclei by a highly specific and sensitive radioimmunoassay.

Swimming stress resulted in a significant reduction of SHIF in the median eminence* (ME) (55±13.4 vs 11.1±2.6 µg/ml respectively). Swimming stress resulted in a significant reduction of SHIF in the median eminence* (ME) (55±13.4 vs 11.1±2.6 µg/ml respectively). Swimming stress resulted in a significant reduction of SHIF in the median eminence* (ME) (55±13.4 vs 11.1±2.6 µg/ml respectively). Swimming stress resulted in a significant reduction of SHIF in the median eminence* (ME) (55±13.4 vs 11.1±2.6 µg/ml respectively).

Several immunohistochemical studies have reported the immunoreactivity of various peptides in hippocampal formation of the rat. Met-enkephalin (M-Enk) and cholecystokinin (CCK) are particularly prominent in the CA2 area of the hippocampus. It was of interest to determine whether these immunoreactive fibers were a) intrinsic to the hippocampus and b) would display a spouting response similar to other neuronal afferents.

Young adult rats were subjected to a variety of surgical procedures which removed all or part of the afferent pathways to the hippocampus. Unilateral lesions of the entorhinal cortex eliminated the major cortical inputs while an aspiration of the fimbria-fornix removed those inputs arising from the contralateral hippocampus and the septal nuclei. Additionally, some animals were given a transaction of the dorsal psalterium, thus removing the crossed tempo-mammonic tract and an aspiration of the ventral, ipsilateral hippocampus was included. Still other animals were injected with 0.8 µg kainic acid-unilaterally which totally destroyed regions CA3, CA4 and portions of CA1 pyramidal cell region. Antisera directed against either M-Enk or CCK were used for immunohistochemical peptide localization utilizing an immunoperoxidase staining technique on 10 µm thick frozen sections fixed with 4% paraformaldehyde in phosphate buffer.

Normal adult animals demonstrated positive staining for both M-Enk and CCK at all levels of the hippocampus with the staining restricted primarily to the CA2 pyramidal cell zone and with minimal staining observed in the subiculum. Adult, lesioned animals demonstrated staining patterns which were indistinguishable from sham-lesioned controls at the light microscopic level. In addition, no other area of the hippocampus was observed to contain positive staining for M-Enk or CCK following the surgical or chemical lesion. However, animals injected with kainic acid at 5 days of age and sacrificed as adults, demonstrated a marked deficit in positive staining for both CCK and M-Enk. Studies have also shown that immature rats (less than 30 days of age) demonstrate a striking difference in staining intensity from adult animals.

The present findings suggest that the immunoreactive fibers containing M-Enk and CCK are intrinsic to the hippocampus and are probably associated with small interneurons in the CA2 region. In addition, these peptide-containing fibers do not appear to sprout outside their normal terminal zones. (Grante MH19691)

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1833 D-ALA9-METHIONINE ENKEPHALAN AMIDE AND MORPHINE ARE ANTI CONVULSANT AFTER CENTRAL ADMINISTRATION IN RATS. F.C. Tortella, A. Cowan, and M.M. Adler, Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Large subcutaneous doses of morphine and several other postulated µ receptor agonists raise the seizure threshold of rats to flurothyl (volatile convulsant; JPET 198:655, 1976). We have reported that D-ala enkephalin amides (D-ala) intracerebroventricularly (icv) to rats now report that both compounds raise the seizure threshold in the flurothyl test.

Male, albino Sprague Dawley rats (initially 180-200 g) were implanted with the icv cannulae aimed at the right lateral ventricle 7 days prior to testing. Morphine (5 and 20 µg), D-ala (10, 40, and 80 µg), or sterile water was injected manually in a 5- or 10-µl volume over a 60-sec time period. Using dose-effect and time-course EEG data previously reported by Tortella et al. (JPET 198:655, 1976), we monitored seizure threshold at the time of peak effect for both compounds: 20 min postinjection for D-ala (all doses) and 75 or 130 min postinjection for 5 and 20 µg morphine, respectively. With groups of 6-9 rats, both doses of morphine increased seizure threshold by 49% and 67%, respectively. Similar effects were produced by D-ala (all doses) and 75 or 130 min postinjection for 5 and 20 µg of D-ala raised seizure threshold above control by 49% and 67%, respectively. Control animals showed a 30% increase in seizure threshold above control. Time-effect studies may reveal even greater anticonvulsant effects. At the time of flurothyl challenge, both D-ala (40 and 80 µg) and morphine (20 µg) induced a profound state of behavioral stupor characterized by exophthalmos, immobility and generalized muscle rigidity. Ketamine (1.0 mg/kg, i.p.) allowed the rats to wake up 15 min before testing, antagonized both the behavioral and anticonvulsant effects.

From these results, we conclude that both morphine and D-ala are convulsant in the rat (when tested at the time of their peak drug effects on EEG and behavior) even though they produce an epileptiform EEG. D-ala is more effective than morphine in this respect.

This work was supported by Grant DA 00376 from NIDA.


A peptide hormone, the eclosion hormone, triggers a 1.5 hour program of stereotyped behavior when injected into silkmoths. When the peptide is added to the isolated CNS of these animals, it evokes the corresponding program of motor activity (Truman, J.W., 1978, J. Exp. Mol. Med. 78: 151-173). A series of 3 methylxanthines were tested for their ability to block the behavioral responses to the hormone. These were theophylline, isobutylmethylxanthine and caffeine; potencies which were indistinguishable from sham-lesioned controls at the light microscopic level. In addition, no other area of the hippocampus was observed to contain positive staining for M-Enk or CCK following the surgical or chemical lesion. However, animals injected with kainic acid at 5 days of age and sacrificed as adults, demonstrated a marked deficit in positive staining for both CCK and M-Enk. Studies have also shown that immature rats (less than 30 days of age) demonstrate a striking difference in staining intensity from adult animals.

The present findings suggest that the immunoreactive fibers containing M-Enk and CCK are intrinsic to the hippocampus and are probably associated with small interneurons in the CA2 region. In addition, these peptide-containing fibers do not appear to sprout outside their normal terminal zones. (Grante MH19691)


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Adult male Sprague-Dawley rats were treated with morphine (3, 10, 40 mg/kg, s.c.) for 3 or 7 days. The effects on substance P-containing neurons were assessed by the Sternberger peroxidase anti-peroxidase (PAP) method and the double bridge PAP method of Vacca et al (J. Histochem. Cytochem. 26: 226, 1978) in the spinal cord and midbrain. Compared with saline and naloxone-treated controls, spinal cord from morphine-treated rats demonstrated increased amounts of substance P (SP) in three regions: substantia gelatinosa (including Rexed's lamina I), the substantia nigra were a) intrinsic to the hippocampus and b) would display a spouting response similar to other neuronal afferents.

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Our laboratories have recently completed a detailed analysis of enkephalin immunoreactive areas in the rat forebrain. We employed untreated animals as well as animals pretreated (48 hrs) with colchicine (to interrupt axonal transport and thus cause neuronal cell bodies to become more apparent) administered (PAP) antibody techniques. We agree well with the description of enkephalin containing areas reported by other laboratories. We have, however, identified many areas of enkephalin-like immunoreactivity which have not been previously reported. These areas include:

- Optic tract: Nervus opticus, hypothalamus, in subcutaneous tissue, in brain stem, in anterior horns of spinal cord and in cerebrospinal fluid.
- Nucleus accumbens: Nucleus accumbens, substantia nigra, nucleus supraopticus, nucleopontica, caudate putamen, in brain stem, in lateral portions of the spinal cord.
- Hypothalamus: Hypothalamus, in anterior hypothalmus, in subfornical organ, and the nucleus paratenialis, among others.

Neuronal cell bodies and fibers are present in these areas. These areas are especially rich in enkephalin-like immunoreactivity, and are thus useful for studying the distribution of enkephalin in the rat forebrain.

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Studies from several laboratories have suggested a physiological role for angiotensin II in drinking behavior and blood pressure response. In this communication we report localization of angiotensin II-like immunoreactivity, by the unlabelled antibody enzyme method, in specific structures or regions of brain. Since it has been shown that blood pressure in spontaneously hypertensive rats (SHR) can be lowered by central injections of angiotensin II antagonists, we have compared the presence of angiotensin II-like material in SHR and their normotensive controls from Wistar Kyoto (WKY) rats. Twelve young adult WKY and SH rats were anesthetized with diethyl ether and perfused via the left ventricle with saline followed by phosphate buffered picric acid-paraformaldehyde, pH 7.4. Vibratome sections, at 50µm and 100µm, were pretreated with triton X-100. The sections were incubated with a 1:1000 dilution of rabbit anti-angiotensin II, titered at 1:95,000 by RIA at 50% binding, for 24 hr at 24°C. The tissue was subsequently incubated with a 1:100 dilution of goat anti-rabbit IgG and a 1:200 dilution of rabbit peroxidase anti-peroxidase. Staining was completely eliminated by the substitution of primary antiserum preabsorbed with angiotensin II. No staining was observed when pre-immune serum was substituted for the primary antiserum.

Angiotensin II-like immunoreactivity was demonstrated in both WKY and SH rats. Cell bodies were observed in supraoptic nucleus, hippocampus and cerebral cortex of both strains. Fiber tracts with darkly staining varicosities were found in the cortex, corpus callosum, septum, striae terminalis, amygdala, hippocampus, fimbria, medial preoptic area, hypothalamus, thalamus, striae medullaris, caudate, putamen, periaqueductal gray and reticular formation of both WKY and SH rats. These fibers, containing angiotensin II-like material, were found in isolation and not in bundles or tracts.

A significant increase in positively staining fibers in the SH rats compared with the WKY rats was demonstrated in the lamina terminalis surrounding the organum vasculosum. This finding, supported by receptor binding studies on angiotensin II previously reported from our laboratory, suggest that the organum vasculosum is an important site for the central regulation of blood pressure in the SH rats.

We are grateful to Detlev Ganten, University of Heidelberg, for supplying the angiotensin II antiserum. JAW is supported by a PMA Foundation Fellowship Award. Supported by NSF grant BNS77-24415 and RSDA to MIP.

Prolactin has recently been reported to regulate the release of striatal dopamine (Perkins and Westfall, Neuroscience 3:59, 1978). As a result of these observations, we have examined the actions of prolactin on the turnover rate of acetylcholine (TRACH) in several brain regions. The striatum and the hippocampus were chosen as areas in which dopaminergic terminals are known to regulate TRACH trans-synaptically while the frontal cortex and parietal cortex were examined as areas in which trans-synaptic dopaminergic regulation of TRACH does not appear to be operative. Prolactin administered intraventricularly (i.v.t.) reduced the TRACH in the striatum and hippocampus while the cortical areas were unaffected. Intraseptal injection of prolactin reproduced the action of the peptide injected i.v.t. on the TRACH in hippocampus but not in striatum. These data suggest that prolactin acts in the septum via changes in the activity of A10 neurons impinging on the cholinergic septal hippocampal cell bodies. In addition, the reduction in striatal and hippocampal TRACH induced by i.v.t. prolactin were nullified by 6-hydroxydopamine lesions of the A9 and A10 dopaminergic cell bodies. In summary, our data suggest that prolactin decreases the TRACH in the hippocampus and striatum by augmenting the release of dopamine from dopaminergic terminals innervating these brain areas.
NEUROPHARMACOLOGY
1844 EFFECT OF PENTYLENETETRAZOL ON MAMMALIAN NEUROMUSCULAR JUNCTION: POSTSYNAPTICALLY MEDIATED DEPRESSION. B. J. Altshul, C. G. Carlson* and C. G. Munial*, Div. Biophysics, Syracuse University and Dept. Physiology, Upstate Medical Center, Syracuse, N.Y. 13210

The synthetic analeptic drug pentylentetrazol (PTE) is a convulsant agent when administered peripherally in sufficient doses. The mechanism of action by which PTE induces epileptic behavior and convulsive seizures is not clearly understood. Research conducted by Boedeker and his colleagues (J. Physiol. 168:100, 1963) demonstrated that PTZ did not significantly affect either prejunctional or postsynaptic inhibition in skeletal muscles. The present experiments will attempt to determine whether PTZ exerts any effect at the mammalian neuromuscular junction.

To test this hypothesis, we performed in vitro intracellular recordings from the diaphragm of young mice (17-23 days of age) were conducted utilizing 3 M KCl glass microelectrodes. PTZ was added to normal mouse Ringer's solution to achieve a final concentration of 100 mM. Dose range: 25-75 %. The spontaneous miniature endplate potential (MEPP) and produced a slight increase in the frequency of MEPP discharge. Following the application of PTE in higher concentrations, muscle contractions induced by low frequency phrenic nerve stimulation were abolished. Details of the experimental design are not presented here, but the results were interpretable. It was observed that PTZ exerted a potent depressive effect on the microelectrode response to nerve stimulation. The effects of PTZ suggest that it has a postsynaptic depression effect at the mammalian neuromuscular junction.

In summary, the results of these experiments indicate that PTZ has a postsynaptic depressive effect at the mammalian neuromuscular junction. Further mechanical and biochemical studies which may elucidate PTZ's effects are currently underway.

(Supported in part by NIH grant 11-1524.)


Guanosine nucleotides activate β-adrenergic receptors (β-AR) and other receptor types. In β-AR systems, this type of regulation appears to reflect the degree of receptor-adenylyl cyclase coupling. Acetylcholine and guanine nucleotides (GTP, GppCH2p) and ATP at concentrations from 1.0 mM.  However, this does not result in any change of specificity of Mg++ and guanine nucleotides to alter ligand binding affinity. These results suggest that PTZ may be exerting a depressant neuromuscular action via some postsynaptic mechanism. Consistent with this interpretation, behavioral experiments indicate that PTZ induces a decrease in response to a constant pulse of iontophoretically applied acetylcholine. The present experiments will attempt to determine whether PTZ exerts any effect at the mammalian neuromuscular junction.

In summary, the results of these experiments indicate that PTZ has a postsynaptic depressive effect at the mammalian neuromuscular junction. Further mechanical and biochemical studies which may elucidate PTZ's effects are currently underway.

(Supported in part by NIH grant 11-1524.)


Monoacylcadaverine's (MCAD) have been suggested recently as possible biological markers for schizophrenia (1). They are novel in vivo antagonists of the dopamine receptors. MCAD has been shown to be present in mammalian brain and blood (3), and is elevated in both tissues during behavioral states (4). The present experiments will attempt to assess the effects of chronic administration of these compounds on sleep-waking behavior.

Adult, CF-1 male mice were implanted with gold wire electrodes for recording the EEG and a modified EKG. Animals were recorded continuously for 6 days under each of the following conditions: lighted, control (administration of saline) and drug administration. A recovery period lasting 6-12 days was also obtained.

The average duration of SWS was significantly decreased below baseline and control durations (p<0.01) in animals receiving monoacylcadaverine at a dose of 100 mg/hr. This decrease in the mean duration of SWS episodes was observed throughout the entire drug period only, whereas night durations remained unchanged. The total amount of SWS was slightly but not significantly increased in the number of SWS episodes. Similar results were seen in animals receiving monoacylcadaverine at a dose of 100 mg/hr., this was not significant. The higher dose of the effect was observed soon after the onset of drug administration. Studies of the effects of monoacylcadaverine on sleep-waking behavior are now in progress.

Supported by USPHS grant NS12482 and NSF grant BNS155127.
CHANGES IN cAMP IN RAT BRAIN. Vernice E. Bates, Robert H. Lenox, G. Jean Kant, and James L. Meyerhoff. Dept. of Medical Neurobiology, Dept. of Psychiatry, University of Vermont, Burlington, VT.

...Our results demonstrate that M excited warm-sensitive cells which are assumed to mediate heat-dissipation responses, and inhibited cold-sensitive cells, which are assumed to mediate heat-conserving responses. The morphine's hypothermic action in the intact animal, and therefore suggest that M lowers Tb by exerting a coherent action on POM- warm and cold-sensitive neurons. Since these effects are mediated by naloxone, the action of morphine on warm- and cold-sensitive cells seems to be mediated by an opiate receptor.


GTP caused a significant(p<0.01)3-fold increase in the inhibitory response of 12 warm-sensitive cells, the other 8 were unaffected. None were inhibited. The FR of 5 cold-sensitive cells was unaffected. One cell was unaffected and none were excited. The temperature-insensitive cells responded variably to M Nitrogen. Four cells were excited, 19 inhibited, and 1 remained unresponsive. These results demonstrate that M excited warm-sensitive cells which are assumed to mediate heat-dissipation responses, and inhibited cold-sensitive cells, which are assumed to mediate heat-conserving responses. The morphine's hypothermic action in the intact animal, and therefore suggest that M lowers Tb by exerting a coherent action on POM- warm and cold-sensitive neurons. Since these effects are mediated by naloxone, the action of morphine on warm- and cold-sensitive cells seems to be mediated by an opiate receptor.


We have previously established (Bédard, P., Barbeau, H. et al., Brain Research, Vol. 169-2, June 1979) that 5 days after transaction of the spinal cord in rats, DL-Strychnylthophenol, 1 mg/kg i.p. induced a powerful muscle contraction in extensor muscles of the tigh. This effect of 5-HT progressively increases until the twentieth day after transaction. It is possible to quantize the response to 5-HT and various agonists of seroton in by measuring the integrated EMG of extensor muscles of the tigh. This effect is not mimicked by drugs acting on other sys tems (Noradrenaline, Dopamine, Acetylcholine and GABA).

Morphyrotranslating hormone (THH) is a tripeptide which, besides its hypophyseotropic action, been shown to be an active substance in the central nervous system of several species. The mechanisms of this action however remains unclear. THH given at a dose of 5 to 10 mg/kg I.P. to rats spinalised at least 15 days previously elicited a strong motor response in both extensor and flexor muscles which is identical to that produced by 5-HTP and 5HT agonists. The effect of THH last between 10 to 60 minutes and can be markedly inhibited by cyproheptadine 10 mg/kg. Glutamic acid 10 mg/kg i.p., one of three amino-acids which constitute THH, did not reproduce the effect of THH. A similar effect was seen in a monkey rendered paraplegic one month previously.

This finding suggest that THH directly stimulates 5HT receptors located on neurons situated in the lumbar spinal cord in both rodents and primates.

(Supported by M.R.C. of Canada)

1853 The differential effects of dorsal horn or ventral horn intraspinal microinjections of Norepinephrine (NE) and 5-Hydroxytryptamine (5HT) on nociceptive C-fiber reflexes (CFR) in the acute decerebrate spinal cat. J. A. Bell* and T. Matsumiya*.

ACUTE DECEREBRATE SPINAL CAT. J. A. Bell* and T. Matsumiya*

LITHIUM (Li) AND TRYPTOPHAN (TP) SYNERGISM IN CENTRAL SERTONERGIC PATHWAYS IN THE MURICIDAL RATS. P. Brade- rick, T. Sanguvi*, V. deP. Lynch* and P. Cervoni*.

1858 The differential effects of dorsal horn or ventral horn intraspinal microinjections of Norepinephrine (NE) and 5-Hydroxytryptamine (5HT) on nociceptive C-fiber reflexes (CFR) in the acute decerebrate spinal cat. J. A. Bell* and T. Matsumiya*.

1862 REPEATED ELECTROCONVULSIVE SHOCK (ECS) AND MORPHINE TOLERANCE: DEMONSTRATION OF CROSS-SENSITIVITY IN THE RAT. Gregory Lucas Belenkyn and John U. Holiday.

John's Univ., Coll, of Pharm., Jamaica, N.Y., 11439 and USP Pharm. Corp., Tuckahoe, NY 10707 (+Lederle Lab, Pearl River, NY 10965).

Therapeutically, the use of Li or TP as thymoleptic agents was confirmed by the effects of Li on TP central serotonin pathways, as studied in rat muricidal (mouse killing) behavior (MB), a model pragmatically employed to screen antidepressant drugs. In the present study, we employed to screen antidepressant drugs.

We have previously shown (Pharmacol., 1978) muricidal Behaviour Antagonism (MBA) by Li and TP, both acutely and chronically, increasing the effects of Li. This cross-sensitivity suggests the physiological changes following repeated ECS and the induction of morphine tolerance share common neurobiological mechanisms.

Panksepp and collaborators (Pharmacol. Biochem. Behav. 2: 213, 1978; Neurosci. Abstr. Abs: 1500, 1979) reported that the narcotic antagonist naloxone disrupted certain mother-infant interactions in various species, thus suggesting that endogenous opiate-like substances may play a role in the mediation of intraspecies social bonding. By use of a long-term delivery system consisting of 1.5 cm beads containing 2 mg of naltrexone base in a polyolactic/glycolic acid copolymer (Dynatech R/D Comp., Cambridge, MA; beads supplied by NIDA), the consequences of chronic blockade of opiate receptors on maternal behavior could be explored.

As soon as feasible after delivery, mothers were implanted subcutaneously with either two naltrexone beads, two sham beads or were left untreated. The naltrexone was 100% effective as judged by its ability to block a ED99 dose of morphine sulfate (i.p., 20 mg/kg) for approximately 30 days. The behavior of sham-planted and untreated mice varied substantially different from one another. However, pup-retrieval times of naltrexone-treated mothers was disrupted and significantly prolonged at least the first 10 days. The pups of untreated mothers gained weight at a significantly slower rate than controls for the first 5 days, after which time treated and controls did not differ. The behavior of the mothers rather than the pups had been altered. Attempts to reverse this effect with naloxone implantation were unsuccessful. These observations support the hypothesis of a role for endorphins in mediating social bonding and do not support one for a noradrenergic component in these behaviors. (Supported by NIMH Grant No. 1-R01 DA02004-01 [NIDA].)

1856 EFFECT OF ALCOHOL INGESTION ON PHYSIOLOGICAL ACTION TREMOR. Scott A. Burgethal*, Robert S. Pozos, Roger V. Petry*, Paul Lefere*. Department of Psychology, University of Minnesota, Duluth, School of Medicine.

During voluntary notion of the hand there is a concomitant high frequency involuntary oscillation called Physiological Action Tremor (PAT). Since it has been reported that alcohol can decrease the amplitude of PAT, Alcohol Research Teram, the present study was undertaken to see if alcohol would also have a similar influence on PAT. If this occurs, insight into the relationship between Physiological and Pathological Action tremors would be obtained.

PAT was recorded using an AVR-250 accelerometer taped on the dorsal side of a styrofoam sandwich which enclosed the hand. EMG's of the extensors and flexors of the wrist were recorded using Beckman bipolar surface electrodes. Accelerometer and EMG signals were recorded onto a Hewlett Packard tape recorder and later analyzed on a PDP-12 computer. Subjects were allowed to drink the alcoholic beverage of their choice until they felt intoxicated. An intoximeter (CIV) was used to quantitate the blood alcohol content of the subjects.

In non-intoxicated subjects, data analysis showed that the maternal behavior of the mothers rather than the pups had been altered. Attempts to reverse this effect with naloxone implantation were unsuccessful. These observations support the hypothesis of a role for endorphins in mediating social bonding and do not support one for a noradrenergic component in these behaviors. (Supported by NIMH Grant No. 1-R01 DA02004-01 [NIDA].)

1857 CORRELATION BETWEEN THE EFFECTS OF CHLOROPROMAZINE ON SPINAL CORD ACTIVITY AND ALPHA-ADRENERGIC BLOCKADE. J.S. Carp* , P. Brigham* and R.J. Anderson (SPON: A. Raines). Dept. of Pharmacology, Brigham and Women's Hospital, Boston, MA.

The effects of chlorpromazine (CPZ) on monosynaptic spinal cord transmission and on adrenergic, cholinergic, and histaminergic receptors were compared in alpha-chloralose anesthetized cats. Monosynaptic transmission between dorsal and ventral roots (L6, L7, or S1) in intact cats was significantly depressed (P < .05) by doses of .0313 to .5 mg/kg of CPZ i.v. This dose-related decrease in transmission was reversible: the removal of beads restored normal behavior. In animals, many normal behavior would first show the disturbed behavior upon removal of the beads. Placing treated mothers with litters untreated parents indicates that the behavior of the mothers rather than the pups had been altered. Attempts to reverse this effect with naloxone implantation were unsuccessful. These observations support the hypothesis of a role for endorphins in mediating social bonding and do not support one for a noradrenergic component in these behaviors. (Supported by NIMH Grant No. 1-R01 DA02004-01 [NIDA].)


The narcotic antagonist naloxone has recently been demonstrated to possess GABA-antagonistic properties (Dingledine, et al., Eur. J. Pharmacol., 42:19-19, 1978). For this reason we decided to compare its effects to a known indirect antagonist of GABA, picROTOXIN. The effects of naloxone (10.0-80.0 mg/kg, i.v. and p.o.), picROTOXIN (0-5 mg/kg, i.v.), muscimol (0.25-0.5 mg/kg, i.v.) and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.,) diazepam (1.0-10.0 mg/kg, i.p.), clonazepam (0.3-3.0 mg/kg, i.m.), and picrotoxin (0.1-0.56 mg/kg, i.m.) were studied alone and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.), diazepam (1.0-10.0 mg/kg, i.p.), clonazepam (0.3-3.0 mg/kg, i.m.), and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.) and picrotoxin (0.1-0.56 mg/kg, i.m.) were studied alone and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.), diazepam (1.0-10.0 mg/kg, i.p.), clonazepam (0.3-3.0 mg/kg, i.m.) and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.), diazepam (1.0-10.0 mg/kg, i.p.), clonazepam (0.3-3.0 mg/kg, i.m.), and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.), diazepam (1.0-10.0 mg/kg, i.p.), clonazepam (0.3-3.0 mg/kg, i.m.), and picrotoxin (0.1-0.56 mg/kg, i.m.) were studied alone and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.), diazepam (1.0-10.0 mg/kg, i.p.), clonazepam (0.3-3.0 mg/kg, i.m.), and picrotoxin (0.1-0.56 mg/kg, i.m.).

Compared the effects of GABA agonists on schedule-controlled responding function, increasing rates of responding without alcohol. These observations support the hypothesis of a role for endorphins in mediating social bonding and do not support one for a noradrenergic component in these behaviors. (Supported by NIMH Grant No. 1-R01 DA02004-01 [NIDA].)
ETHANOL ORAL SELF-ADMINISTRATION IN NAÏVE AND TOLERANT DROSOPHILA MELANOGASTER. S. S. Chawla\textsuperscript{a}, N. M. Perron\textsuperscript{b}, and C. Dado\textsuperscript{c}. Unité de Recherche sur L’Abus des Drogues et de l’Alcool, Hôp. St-Français d’Assise, Dpt. Pharmaco., Faculté de Médecine\textsuperscript{d} et Dpt. Biol., Faculté des Sciences\textsuperscript{e} of Université Laval, Québec, Canada.

A method to measure the microvolumes of ethanol solutions self-administered orally by Drosophila melanogaster Meigen is described. The apparatus consists in a battery of eight experimental units, each one composed of a plastic chamber and an injection circuit. The net amount of ethanol taken has been examined in various experimental conditions: ethanol alone, ethanol-sucrose solutions at various concentrations and ethanol-sucrose cube. The net values for each day were obtained by computing the difference between gross values and evaporation per day figures. The data obtained in naïve drosophila show that the amount of self-administered ethanol is directly related to the concentration of ethanol; the consumption of sucrose solutions is inversely proportional to the concentration of sucrose. Observations on 14 days showed that the lethality increased with the raise in the concentration of ethanol and decreased with the raise in the concentration of sucrose (sucrose-water solutions 0.5-3%).

Experiments have been carried out also on adult drosophila which have been exposed to ethanol during their entire life cycle. The preliminary results suggest that this new self-administration method could be envisaged for the study of the experimental dependence on ethanol and other addictive psychotropic drugs.

THIOHETHANIL ETANOMIERS: PHARMACOLOGICAL ACTIVITY. H. DIF Christensen, N.C. Goad\textsuperscript{a}, Philip Abraham\textsuperscript{a} and F. J. Carroll\textsuperscript{a}. Dept. Pharm., OUHSC, Okla. City, OK 73190 and RTI, Research Triangle Park, N.C. 27709.

Quantitative pharmacological differences exist between the optical antipodes of barbiturates. Racemic thiocarbamoyl in humans has a fast metabolism rate, 25% per hour, but possesses undesirable side effects of hiccoughs and twitching of extremities (Market et al., Anes. 29:1159 (1968)). The relative anesthetic potency, toxicity, and incidence of side effects as a function of the configuration of thiocarbamoyl was investigated in Charles River, CF-1 male mice. The AD\textsubscript{50} (loss of righting reflex), and LD\textsubscript{50} values with 95% confidence limits after intraperitoneal administration are as follows:

<table>
<thead>
<tr>
<th>Stereoisomer</th>
<th>Potency Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD\textsubscript{50}</td>
<td>(24.5-27.2)</td>
</tr>
<tr>
<td>Racemic</td>
<td>(1.06-1.48)</td>
</tr>
<tr>
<td>(+)</td>
<td>(1.09-1.24)</td>
</tr>
<tr>
<td>(-)</td>
<td>(1.14-1.22)</td>
</tr>
<tr>
<td>LD\textsubscript{50}</td>
<td>(25.9-28.8)</td>
</tr>
<tr>
<td>Racemic</td>
<td>(1.02-1.22)</td>
</tr>
<tr>
<td>(+)</td>
<td>(1.05-1.23)</td>
</tr>
<tr>
<td>(-)</td>
<td>(1.06-1.40)</td>
</tr>
</tbody>
</table>

There were no significant differences in onset, 3.68 ± 0.40 min., and duration of anesthesia, 12.11 ± 1.92 min., when the compounds were administered at the respective AD\textsubscript{50} values to sets of 40 mice. Although the incidence of tremor on recovery was less for the (+) stereoisomer than (-) or racemic mixture, it is still significant compared to other alkyl-substituted barbituric acid rates. When administered intravenously, the onset and potency are dependent upon the rate of injection, because of the high lipid solubility.

This study in addition to others with barbiturate enantiomers suggests that binding to receptor(s) is determined by the absolute configuration.

Our previous findings that CAMP dose-relatedly regulates duration of narcosis led us to investigate whether brain CAMP concentration is altered by pentobarbital. CAMP metabolism in either whole or specific brain structures failed to establish a relationship between CAMP brain content and state of narcosis. Nevertheless, we planned to investigate a putative anesthetic effect on CAMP metabolism in order to test whether either catabolism or degradation is altered by barbiturates. Male Sprague-Dawley rats were anesthetized with intraperitoneal sodium pentobarbital (200mg/kg); controls were untreated. Following sacrifice by either microwave radiation (MR) (5 sec. exposure) or decapitation (D), brains were immediately removed, homogenized in 4% perchloric acid (3ml/400mg of wet brain), centrifuged, neutralized and filtered. After D, brain cortex slices were incubated under aerobic conditions in Krebs-Ringer bicarbonate buffer pH 7.4 (30°C) with or without pentobarbital (10-6M). Sequential aliquots of incubation mixture and brain extract samples were analyzed with our newly devised high-performance liquid chromatographic method (Cohn et al., Pittsburgh Conference on Analytic Chemistry and Applied Spectroscopy, p. 520, 1979). Contrary to previous reports that in mammalian brain major metabolic products of ATP and CAMP are adenosine and adenosine triphosphate, our findings in untreated rats sacrificed by MR showed smaller concentrations of the two metabolites and much higher accumulations of deamination products inotose and hypoxanthine, which are presumably derived from adenosine. The shift in CAMP metabolism produced by pentobarbital while similar in D was more pronounced than that induced by D. In-vitro incubation studies revealed that pentobarbital reversibly inhibits deamination of 5'AMP, leading to adenosine accumulation. This phenomenon is not specific to the use of pentobarbital or to chromatographic evidence that barbiturates inhibit deamination in CAMP metabolic pathways provides a molecular basis for the 50-year-old observation that anesthetics decrease brain ammonium concentrations. That pentobarbital increases adenosine, which allegedly regulates cerebral blood flow, may partially explain the barbiturate-induced therapeutic usefulness against ischemia and stroke. Recently, it was proposed that inosine and hypoxanthine are endogenous ligands of benzodiazepine receptors. Our earlier finding that, like barbiturates, diazepam-induced narcosis is dose-relatedly shortened by CAMP suggests that diazepam may also duplicate barbiturates' specific inhibition of deamination and subsequent lowering of the two metabolites' concentrations. Future investigations will verify our hypothesis. Supported by DAO0605.

LESIONS OF THE SUBSTANTIA NIGRA REVEAL BENZODIAZEPINE RECEPTORS WITH AND WITHOUT γ AMINOBUTYRIC ACID RECEPTOR LINKAGE. Tomasino and Capt. Agu Pascual. Biological Psychiatry Branch, NIMH, Bethesda, MD 20010 (SPONSOR: L. K.Y. Ng).

Four weeks following the destruction of cell bodies in the substantia nigra by injection of 0.5 mg of kainic acid (specific [2H]diazepam binding was reduced 60% in the lesioned side compared to the unlesioned control side. The ability of γ-aminobutyric acid (GABA) (10 µM) to enhance [3H]diazepam binding was significantly reduced on the lesioned side. A parallel, but smaller (40%) decrease on the lesioned side was also observed for [3H]muscimol binding. The restriction of the [3H]diazepam binding to the specific input pathway as it was also observed when the pyramidal cells were driven antidromically by stimulation of the alveus. The additional observation that diazepam binding is not altered by a decrease in recurrent inhibition upon the CAI neurons, a presumed GABA-mediated effect. The morphine effect was indeed reversed by the addition of GABA (1 mM), while the effect of the GABA agonist, picro-toxin (2.5 µM), a GABA antagonist, mimicked the effect of morphine. These data are evidence for a specific, primary effect of morphine in the hippocampus, which at the cellular level may consist of a selective depression of the interneurons normally mediating recurrent inhibition to the CAI pyramidal cells, resulting in net excitation of the latter.
1867 DEVELOPMENT OF TOLERANCE TO OPIATES AND OPIOID PEPTIDES IN CULTURES OF MOUSE SPINAL CORD WITH ATTACHED DORSAL ROOT GANGLIA. Stanley M. Crain, Be congestion, Tana Finno, * and Eric J. Simon*. Depts. of Neuroscience, Physiol. and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461; (E.J.S.) Dept. of Medicine, New York University College of Medicine, New York, N.Y. 10016.

Acute opiate-depressant effects on sensory-evoked dorsal-sensory network response of organotypic explants of fetal mouse spinal cord with attached dorsal root ganglia (in vitro) (133, 137; 152, 157) disappeared after chronic exposure to analgesic levels of morphine (1 µM) for > 2-3 days in culture at 35°C. (Drug exposure was begun at 4 weeks after transplantation.) Characteristic dorsal cord responses could then be evoked by dorsal root ganglion (DRG) stimuli in the absence of morphine -- even at acute increase in the concentration (100-fold). Tolerance also developed after chronic exposure of cord-DRG explants to low concentrations (ca. 0.01 µM) of the enkephalin analog, [d-Ala2, Me-Phe4, Leu5]-enkephalin (Sandoz 33-824). The latter cultures showed cross-tolerance to met-enkephalin and to the Sandoz opioid peptide (33-824).

In cultures where endogenous neurotransmitters were incubated at lower temperature, 20°C, during exposure to 1 µM morphine for as long as 7 days. (Control 2-week-old cultures incubated at regular temperature at 20°C showed no significant electrophysiological deficits.) Furthermore, development of tolerance was partly antagonized by raising the Ca++ concentration of the medium to 10 µM during chronic exposure to 1 µM morphine. The latter result is in agreement with our acute experiments showing that the depressant effect of opiates and opioid peptides on dorsal cord responses is markedly reduced in the presence of high Ca++ (the culture medium (Crain et al., Br. Res. 152, 157). The sensory-evoked dorsal-sensory population responses in tolerant cultures developed usually large amplitudes during maintenance in 1 µM morphine or after acute introduction of naloxone. Experiments chronically exposed to morphine continued to show evidence of tolerance for about 2 days after transfer to drug-free culture medium, but characteristic sensitivity to opiates returned within the subsequent few days. Organotypic cord-ganglion cultures provide, therefore, a valuable model system for analyses of cellular mechanisms underlying development of opiate tolerance and dependence in mammalian CNS tissues under flexible, controlled in vitro.

Supported by research grants to S.M.C.: DA-0203 from NIDA and NS-12405 from NIMCOs; to E.J.S.: DA-0017 from NIDA.


Systemic d-amphetamine (d-A) can release dopamine (DA) from neuronal terminals in the ventral tegmental area (VTA) and other mesolimbic DA systems, and may therefore disrupt the function of monoaminergic neurotransmission. In addition, d-amphetamine produces hyperactivity, increased feeding, increased reactivity to aversive stimuli, and an increased level of DA release in the VTA. These effects are thought to be mediated by d-A-induced activation of DA receptors in the VTA, which may result in increased DA release, increased DA synthesis, and increased DA uptake. However, the precise mechanisms by which d-amphetamine induces hyperactivity and DA release in the VTA are not yet fully understood.

In our experiments, we have examined the effects of d-A on DA release and DA synthesis in the VTA using in vivo microdialysis techniques. We have found that d-A induces a significant increase in DA release in the VTA, which is accompanied by an increase in DA synthesis. These effects are blocked by the DA receptor antagonist, haloperidol. We have also found that d-A-induced hyperactivity is accompanied by an increase in DA release in the VTA, which is also blocked by haloperidol. These findings suggest that d-A-induced hyperactivity is mediated, at least in part, by activation of DA receptors in the VTA.

Supported by VA 1680, N.I.H. NS 06233 and NS 13101.
ELECTROPHYSIOLOGICAL CORRELATES OF NORMAL AND HALOPERIDOL-INDUCED INMObILITY IN RATS. Marc De Ryck* and Philip Leliotte. Psychol. Dept., Univ. of Illinois, Champaign, Ill. 61820.

Weakened immobility rates in rats chronically treated with bipolar electrodes in somatosensory cortex (SSC) and hippocampus (H), was accompanied by low voltage fast activity (desynchroniz­ation, DESYNC) and large amplitude irregular activity in H. However, between periods of weakened immobility and prior to the onset of slow wave sleep (SWS)—i.e., large amplitude slow waves in SSC and H)—transient hypersynchrony mixed with DESYNC appeared in SSC. This activity consisted of spindle-shaped bursts of biphasic spike or spike-wave discharges with frequencies of 7-10 spikes/sec and durations of 1-30 sec. Spikes amplitudes (0.3-2.2 mV), which always exceeded wave amplitudes (1.2-7 mV), were 2-13 times larger than the background DESYNC. SSC spiking spindles occurred in apparently awake but immobile rats and were associated with head bobbing in phase with the spike discharges together with fine whisker tremor. SSC spiking spindles in rats may represent an EEG correlate of a distinct immobility state occurring between wakeful immobility and SWS.

Similar “twilight states” have been reported in cats and monkeys by Kramar, Rougeul and their associates.

5-5 mg/kg haloperidol (HAL) produced catalepsy/akinesia accompanied by a dose-dependent increase in occurrence of SSC and C large amplitude slow waves similar to those observed in normal SWS. Thus, at these dosages, HAL predominantly produces somnolent immobility. At higher dosages (10-15 mg/kg), this SWS pattern was replaced, often abruptly, by almost continuous SSC spiking spindles (e.g., during 65-95% of a 30 min. period).

Cholinergic agents (.5 mg/kg physostigmine, 8 mg/kg arecoline) completely abolished HAL-induced SSC spindle activity and produced continuous DESYNC. Likewise, anticholinergic drugs (10 mg/kg atropine, .5 mg/kg scopolamine) abolished HAL-induced SSC spindles, but replaced them by large amplitude slow waves. (synchronization, SYNCH). When anticholinergic and cholinergic agents were sequentially injected in rats with HAL-induced SSC spindles, the shift from desynchrony to synchronization occurred as a stage in which spiking spindles temporarily reappeared. In contrast to their effect on HAL-induced SSC spiking spindles, neither cholinergic nor anticholinergic agents suppressed petit mal or grand mal seizures associated with very high dosages of HAL (20-45 mg/kg). Thus, to the extent that SSC spiking spindles represent a dopaminergic-cholinergic imbalance, their reversal by both cholinergic and anticholinergic drugs suggests that this EEG correlate of normal immobility and HAL-induced akinesia depends on a narrow range of dopaminergic-cholinergic interactions. Supported by NIH Grant BOL NS 11671.

LEAD-INDUCED HYPERACTIVITY IN MICE AND BLOOD-BRAIN BARRIER FUNCTION. Floyd R. Domer and Carlos Wolf*. Dept. of Pharmacology, Tulane Univ. School of Medicine, New Orleans, La. 70112.

The offspring from CD-1 mice were exposed to 0.5% sodium or .5-5 mg/kg arecoline) abolished HAL-induced SSC spindles, but replaced them by large amplitude slow waves. (synchronization, SYNCH). When anticholinergic and cholinergic agents were sequentially injected in rats with HAL-induced SSC spindles, the shift from desynchrony to synchronization occurred as a stage in which spiking spindles temporarily reappeared. In contrast to their effect on HAL-induced SSC spiking spindles, neither cholinergic nor anticholinergic agents suppressed petit mal or grand mal seizures associated with very high dosages of HAL (20-45 mg/kg). Thus, to the extent that SSC spiking spindles represent a dopaminergic-cholinergic imbalance, their reversal by both cholinergic and anticholinergic drugs suggests that this EEG correlate of normal immobility and HAL-induced akinesia depends on a narrow range of dopaminergic-cholinergic interactions. Supported by NIH Grant BOL NS 11671.

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SYSTEMIC OR LOCAL ADMINISTRATION OF LSD CAUSES COMPLEX CHANGES IN THE DISCHARGE PATTERN OF VISUAL CORTEX NEURONES WHEN THESE ARE ACTIVATED BY PHYSIOLOGICAL OPTICAL STIMULATION. Rose & Horn, 1977, Exp. Brain Res. 27, 21; Hilley et al., 1977, Neurosci. Abstr. 3, 562; Fox & Dray, 1979, Brain Res. 161, 107). To determine whether these changes are related to the hallucinogenic properties of LSD the actions of this drug have been compared with the supposed non-hallucinogenic analogue 2-bromo-LSD (BOL). Data were collected from single neurons in the striate cortex of anaesthesia­nized, immobilized cats. Drugs were administered intravenously or locally by microelectrodes. Visual stimulation usually consisted of an optimally oriented illuminated light bar moving back and forth across the receptive field. Systemic LSD either enhanced or depressed visually evoked responses. These effects were clearly dose related, enhancement being observed with low doses (0.1-10 ug/kg) and depression more frequently at higher doses (10-25 ug/kg). Accompanying this were changes in neuronal receptive field properties, direction selectivity and unstimulated background discharges. Such effects were observed on cells with both simple and complex receptive fields. Electrophoretic LSD produced similar dose dependent effects suggesting a direct action of LSD on the visual cortex. Unexpectedly, systemic BOL (10-25 ug/kg) also produced similar effects to LSD. However, whole nerve action potentials suggested that it was some 20-100 times less active in producing enhancement of visually evoked activity than its hallucinogenic analogue. Supported by grants NIDA#DA01458 and 5-T01-MH-08394-13

REGULATION OF B-ADRENERGIC RECEPTORS AND cAMP ACCUMULATION IN VITRO IN SLICES OF RAT CEREBRAL CORTEX. Mark D. Dibner and Perry H. Molinoff. Dept. of Pharmacology, Univ. of Colorado Med. Cent., Denver, CO 80262.

The effects of incubating slices of rat cerebral cortex with the B-adrenergic agonist isoproterenol (ISO) on B-adrenergic receptor density and on catecholamine-stimulated cAMP accumulation were investigated. Exposure of brain slices to ISO (0.1-10 µM) caused a decrease in receptor density (as measured by Scatchard analysis of IHYP binding). The effect of ISO was complete by 3-5 min and had an IC50 of 10 µM. In contrast, cAMP accumulation decreased by approximately 75% as measured by a protein binding assay. The effect of ISO on cAMP accumulation was more rapid (complete by 0.5 min) and had an EC50 of approximately 0.01 µM. Thus, incubation with ISO under appropriate conditions led to decreases in B-adrenergic receptor mediated cAMP accumulation without changing receptor density. The effects of ISO on receptor density and on ISO-stimulated cAMP accumulation were blocked by coincubation with the B-adrenergic receptor antagonist sotalol. The cAMP effects appeared to be receptor specific since PGE, stimulation of cAMP was not decreased following incubation with ISO nor was IHYP binding decreased following incubation of slices with PGE. Incubation with ISO did not significantly alter the IC50 for displacement of IHYP binding by either agonists or antagonists. Lastly, the observed decreases in B-adrenergic receptor numbers could be slowly reversed by reincubation of slices in the absence of ISO or rapidly reversed by incubation of homogenates with guanine nucleotide before binding.

Supported by the USPHS NS 12529 and NS 09199 and NHF fellowship NS 05714.


Systemic or local administration of LSD causes complex changes in the discharge pattern of visual cortical neurons when these are activated by physiological optical stimulation (Rose & Horn, 1977, Exp. Brain Res. 27, 71; Hilley et al., 1977, Neurosci. Abstr. 3, 562; Fox & Dray, 1979, Brain Res. 161, 107). To determine whether these changes are related to the hallucinogenic properties of LSD the actions of this drug have been compared with the supposed non-hallucinogenic analogue 2-bromo-LSD (BOL). Data were collected from single neurons in the striate cortex of anesthetized, immobilized cats. Drugs were administered intravenously or locally by microelectrodes. Visual stimulation usually consisted of an optimally oriented illuminated light bar moving back and forth across the receptive field. Systemic LSD either enhanced or depressed visually evoked responses. These effects were clearly dose related, enhancement being observed with low doses (0.1-10 ug/kg) and depression more frequently at higher doses (10-25 ug/kg). Accompanying this were changes in neuronal receptive field properties, direction selectivity and unstimulated background discharges. Such effects were observed on cells with both simple and complex receptive fields. Electrophoretic LSD produced similar dose dependent effects suggesting a direct action of LSD on the visual cortex. Unexpectedly, systemic BOL (10-25 ug/kg) also produced similar effects to LSD. However, whole nerve action potentials suggested that it was some 20-100 times less active in producing enhancement of visually evoked activity than its hallucinogenic analogue. Supported by grants NIDA#DA01458 and 5-T01-MH-08394-13
SLOW POTENTIATION OF CA1 HIPPOCAMPAL SLICE FIELD POTENTIALS AND ACUTE EFFECTS OF LOW-DOSE ETHANOL. Dominique Durand and Peter Carlin. Neurobiology Lab of the Addiction Research Foundation Clinical Institute, Flagler Neuroscience Unit, Toronto Western Hospital, University of Toronto, Toronto, Canada.

Field potentials were recorded from CA1 hippocampal neurons in the in vitro transverse hippocampal slice preparation. Orthodromic stimulation of the stratum radiatum at low frequency (.1 to .2 Hz) induced a slow but steady increase in the size of the field potential (2 to 10 fold in 30 to 60 min.). Input/output (I/O) curves of the population spike recorded at 10 min. intervals over a 30 to 60 min. period showed a definite increase in amplitude and a shift to the left. This decrease in the threshold of the response cannot be attributed to an increased afferent volley since the size of the presynaptic spike remained constant.

Because of this slow potentiation, efforts of low-dose ethanol (50 nM to 100 nM) were difficult to assess. However, after stabilization of the response, ethanol was added to the bath. Ethanol at 100 nM consistently produced a reversible depression of the field potential of 25%. 50 mM had different effects: in a total of 13 slices, 7 showed a depression (5 to 20X) of the population spike (5 of which were accompanied by acute tolerance i.e., return to baseline during ethanol perfusion); 4 slices showed no changes; 2 slices were accompanied again by acute tolerance; 2 slices did not show any effects. The time course of the peak ethanol response was between 15 to 30 min. This response returned to baseline during the ethanol infusion after another 15 to 30 min. period.

These depressing effects of ethanol were reversible within 30 min. by switching back to the control solution and were accompanied by an increase threshold as shown by a shift to the right of the input/output curves recorded during the ethanol perfusion period. Because the ethanol-induced increase in the size of the population spike was correlated with shorter periods of stimulation before stabilization, we feel that this response could also be attributed to further potentiation of the field potential, the later "tolerance" to this increase being then the depressing effects of ethanol.

These initial results suggest that clinically relevant doses of ethanol (50 to 100 nM) are effective in vitro but further experiments are necessary to understand these acute physiological changes.

(supported by the Addiction Research Foundation and the Medical Research Council of Canada)


Several hypothesis relating Dopamine system mechanisms to the effects of dopaminergic agonists and antagonists have been presented for experimental models of clinical disease ranging from dyskinesia to psychosis. Most frequently chronic drug induced behavioral changes and changes in stereotypy (e.g., hyperactivity, stereotypy) are considered main in terms of post-synaptic receptor changes rather than the interaction of neuronal with synaptic mechanisms. This report will review data from several studies in which dopaminergic system sites, e.g., either unilateral substantia nigra and caudate are chronically treated with dopamine agonists or antagonists instilled via cannula connected to Alza osmotic minipumps, then tested for post-treatment responsivity to apomorphine and amphetamine. For example, preliminary data analysis indicates that chronic amphetamine into the caudate leaves a residual state where animals rotate contralateral following i.p. amphetamine, yet show no rotational preference to apomorphine. This implies a pre-synaptic mechanism. On the other hand, chronic L-Dopa instillation into the substantia nigra renders animals that rotate ipsilaterally to amphetamine which is in keeping with a sub-synergistic neuronal dopaminergic receptor mediating an inhibition of firing rate. In general, our results are consistent with a significant pre-synaptic role for residual chronic drug effects.

SPECIFIC PHOSPHOPROTEINS IN THE FUNCTION OF OPIATE RECEPTORS. Yigal H. Ehrlich, Leonard G. Davis and Peter Keen.

Department of Pharmacology, School of Medicine, University of California, San Francisco, CA 94132.


Anticholinergic agents such as Cogentin (COG) and Artane (ART) are often coadministered with antipsychotic drugs to reduce extrapyramidal side effects. The anticholinergics are thought to result from a shift in the functional balance between acetylcholine (ACh) and dopamine (DA) in the striatum (ACH:DA). It has recently been suggested that chronic L-DOPA instillation into the nigrostriatal system may also alter the functional balance between dopamine and acetylcholine (DA: ACh). In order to determine whether this occurs, the authors (1975) to extract stereospecific opiate-binding-sites from neostriatal membranes. When synaptic membranes were first incubated with Y-32P-ATP and then extracted with Brij-36, close to 100% of the phosphorylated protein F and some of the H co-extracted with the opiate receptors. Moreover, the Brij extract and the membrane-residue retained phosphorylated activity. Endogenous phosphorylation of protein F was in evidence only in the extract. Chroma­
tographically pure extract of protein F (0.05-0.1 mg/ml) yielded macromolecular protein complexes that contained protein-bound [3H]tetorphine and the endogenous phosphorylation systems. These complexes, which were shown to be opiate binding domains (OBD) (Ehrlich et al. 1975) to distort the opiate binding sites, caused selective inhibition of the endogenous phosphorylation of F and H, without affecting the specific binding properties of the opiate receptor to synaptic membranes. Finally, opiate-inhibited phosphorylation of F and H was accompanied by altered activity of adenylate cyclase from the same preparations. Preliminary experiments indicate possible cause-effect relationships between these systems. The results suggest that the function of opiate receptors may involve complex interactions between opioid peptides, their binding sites, and membrane-bound systems that phosphorylate specific phosphoproteins. Supported in part by a grant from the Epilepsy Foundation of America to Y.H.E., and by intramural funds from the MIP.
ASSESSMENT OF ANTICONVULSIVE EFFECT OF DIAZEPAM IN PENTOBARBITAL DEPENDENT MICE. B. A. Flint and I. K. Ho, Dept. Pharmacol. & Toxicol., Univ. Miss. Med. Ctr., Jackson, Miss. 36196. Results of the administration of the threshold for pentylenetetrazol (PTZ) or audiogenic induced convulsions in pentobarbital dependent mice was assessed. Male ICR mice were rendered dependent on pentobarbital by the subcutaneous implantation of a 75-mg pentobarbital pellet (acid form). Control animals received placebo pellets. After three days of pellet implantation the pellets were removed and animals were administered saline or diazepam, 1 mg/kg, i.p. Six hours following pellet removal CNS hyperexcitability was evaluated by two different methods. At least three different doses of PTZ were administered s.c. to separate groups of animals. The percent of mice having a tonic convolution within five minutes was recorded. A second method used for assessing CNS hyperexcitability by placing them in an audiogenic seizure box equipped with a two inch in diameter bell. The percent of mice having a tonic convolution within thirty seconds after the initiation of the audiogenic stimulus was recorded. Diazepam was observed to increase significantly the ED50 for PTZ and audiogenic induced convulsions. The involvement of the GABA system related to the observed increase in ED50 for PTZ in diazepam administered animals was evaluated. The GABA levels in pentobarbital dependent animals was significantly lower than those of the placebo treated mice. In GABA was also noticed to occur in dependent mice that convulsed after the administration of PTZ as compared to those that failed to convulse. The GABA level in diazepam treated pentobarbital dependent mice was significantly elevated as compared to non-treated pentobarbital dependent animals. Also, the activity of L-glutamate-decarboxylase measured in dependent mice that convulsed was significantly lower than those of non-convulsed dependent mice. Thus, it was shown that diazepam is effective in increasing the ED50 for PTZ. A functional induced convulsions in pentobarbital dependent animals. Also, an observed decrease in GABA levels seemed to be associated with the PTZ and audiogenic induced convulsions in pentobarbital dependent mice. Therefore, it is possible that the GABA system is involved in the increased hyperexcitability observed at six hours following pentobarbital dependent mice (Supported by Grant DA-01403 from the National Institute on Drug Abuse).

MORPHINE ENHANCES AND DIAZEPAM SUPPRESSES THE NEUROTOXICITY OF SYSTEMICALLY ADMINISTERED KAINIC ACID. Terry A. Fuller and John M. Olney. Washington University, School of Med., St. Louis, MO 63110. Kainic acid (KA) induces seizures, convulsions and acute neuronal necrosis in various brain regions, particularly the hippocampus and olfactory cortex. Since KA is a broad spectrum neurotoxin observed in rats undergoing naloxone-precipitated withdrawal from opiates, we explored in a previous study, the effects of naloxone pre-treatment on KA-induced tissue destruction. Here we report the anticonvulsant and neuroprotective effects of diazepam on KA induced seizures and blocks the "distant" hippocampal degeneration associated with local injections of KA into the amygdala. We report that morphine augments and diazepam suppresses both the convulsions and brain damage effects of systemically administered KA.

When adult male Sprague-Dawley rats were given 7 mg/kg KA subcutaneously (sc), only 1 of 14 (7%) exhibited convulsions and sustained acute damage to the hippocampus and olfactory cortex. When the same dose of KA was preceded by injection of morphine, 10 mg/kg sc, 10 of 10 animals (100%) convulsed and sustained hippocampal and olfactory cortical damage. When 29 rats were injected with 12 mg/kg KA sc, 27 (93%) exhibited convulsions and the typical pattern of brain damage, but when this dose of KA was preceded by 20 mg/kg diazepam sc, the pattern of brain damage was characteristic of morphine damage and only detectable in one brain region (CA3 hippocampus). The severe convulsions typically observed in rats treated with 12 mg/kg KA were eliminated and the incidence of NS was not reduced by diazepam pre-treatment.

Our findings suggest that KA-induced convulsions and brain damage are closely associated phenomena; morphine augments both effects and diazepam suppresses both. The suppressant action of diazepam differs from that of naloxone in that KA is not influenced by morphine but not diazepam. Additional research will be required to adequately clarify the mechanisms and receptors involved in the modification of KA neurotoxicity by these agents. Support by NIH grants NS-15360, DA-005556, a Huntington's Chorea Fdn. grant and RSD Award MH-38894 (JMO).

PHYSICAL DEPENDENCE TO FK-33,824 [Tyr-DAla-Gly-MePhe-Met-(0)-ol], A SYNTHETIC METHIONINE ENKYPHALIN ANALOGUE, IN THE CHRONIC SPINAL DOG. P. E. Gilbert* and D. R. Jasinski. NIDA Addiction Research Center, Lexington, KY 40504. FK-33,824 is a pharmacologically active peptide with some pharmacologic actions in the chronic spinal dog that resemble those of morphine (Gilbert and Jasinski, Fed. Proc., 1979). To confirm that FK-33,824 is a direct addictant, a precipitation study was done in the chronic spinal dog. FK-33,824 was infused through an indwelling jugular catheter every 4 hr in a dose of 0.35 mg/kg/infusion in five chronic spinal dogs. The animals were stabilized at this dose of FK-33,824 for 2 weeks prior to the beginning of precipitation and suppression experiments. Throughout the study, 1 to 5 min after each drug infusion the dogs vomited. (This action of FK-33,824 was also observed in other dogs that received the drug only one time in acute studies.) Tolerance did not develop to this effect of FK-33,824, nor could the vomiting be blocked by the prior administration of 1.0 mg/kg of naltrexone in nondependent dogs. Naltrexone precipitated an abstinence syndrome in animals receiving FK-33,824 chronically. This abstinence syndrome resembled the naloxone precipitated abstinence syndrome in morphine-dependent dogs. The most prominent signs of precipitated abstinence were stepping, salivation, mydriasis, tachycardia and tachypnea. Naltrexone was only about 1/8 as potent in precipitating an abstinence syndrome in FK-33,824-dependent dogs as it was in morphine-dependent animals. The dogs began to show signs of withdrawal abstinence approximately 4 hr after their last infusion of FK-33,824. Withdrawal from FK-33,824 had a more rapid onset (10-12 hr) than withdrawal from morphine. Both FK-33,824 and morphine suppressed withdrawal in 12-hr abstinence FK-33,824-dependent dogs. In contrast, the chronic administration of FK-33,824 produces physical dependence in the dog. Additionally, the emetic effects of FK-33,824 are probably due to interactions at a non-opioid receptor since this action of FK-33,824 is not antagonized by doses of naltrexone, which precipitates an abstinence syndrome in dogs receiving FK-33,824 chronically and antagonizes the cardiovascular and flexor reflex depression produced by single doses of FK-33,824.
1883 DEVELOPMENT OF OPIATE MECHANISMS IN THE GUINEA PIG SMALL INTESTINE IN RELATION TO THE ONGOING OF NEURONS KNOWN TO BE COMPONENTS OF THE ENTERIC NERVOUS SYSTEM. Alan R. Gintzler*, Taube P. Rothman and Michael D. Gershon. (Spon. K. Pfenninger.) Dept. of Neur. of the enteric nervous system. At 25 days gestation, neurons and a transmitter mechanisms of neurons known to be components of the intestine has been studied with reference to the development of precursors, however, are probably present prior to 25 days. Putative serotonergic precursors were recognized by their uptake of 5-HT into cell bodies as early as day 20. Adrenergic innervation appeared gradually between days 32 and 48. Functional innervation of the longitudinal layer of smooth muscle was established much later in ontogeny. Spontaneous tone and a tetrodotoxin-sensitive, 5-HT-induced relaxation of the muscle were detected at day 42 (neither norepinephrine nor ATP mediate this effect). Cortical EEG electrodes and a jugular catheter, were prepared Rats chronically prepared with hippocampal microelectrodes, for cortico-cortical EEG electrodes and a jugular catheter, were prepared. Picrotoxin pretreatment increased unit firing rate above baseline levels, produced a reduction in heart rate and induced epileptiform (spike and wave) activity in the frontal cortex. The subsequent administration of amphetamine produced an inhibition of firing which did not differ in degree from that obtained without pretreatment, although the final firing level achieved was considerably higher than that in the absence of any pretreatment. Cortical EEG was briefly normalized to low amplitude fast activity before reverting back to spike and wave activity, and heart rate reduction appeared again higher than in the absence of pretreatment. Picrotoxin pretreatment reduced increases in firing rate, yielded an essentially activated EEG, but did not decrease heart rate appreciably. The administration of amphetamine for those units enhanced by the pretreatment brought about a quantitatively similar inhibition in rate to that obtained in the absence of any pretreatment although the firing level was again higher than in the absence of pretreatment. Those units inhibited by the pretreatment did not appear to show any further rate reduction following amphetamine. EEG following ethanol showed little change from the activated mode, while heart rate also remained unaffected. Control experiments with neostigmine resulted in abrupt increases in rate, hemoglobin, and peripheral blood pressure. These data indicate that the manipulation of hippocampal responsiveness either by interfering with gaba-mediated inhibition or by augmenting cholinergically-mediated excitation does not alter the degree of firing depression produced by ethanol. (Supported by the Addiction Research Foundation of Ontario).


Recent evidence has implicated the involvement of striatal cholinergic (Ch) interneurons in a feedback regulation of activity in the nigrostriatal dopamine system. Since Ch motor stimulants are believed to act primarily through their effects on central DA activity, Ch modulation of DA neurons might play a role in mediating the effects of the stimulant on behavior. The present study examined the effects of altered Ch activity on behavioral stereotypy resulting from treatment with the direct receptor stimulant amphetamine and effects resulting from agents which alter pre-synaptic DA release (amphetamine and methyphenidate). Altered feedback regulation of pre-synaptic DA activity would be expected to interfere with the effects of those stimulants acting pre-synaptically to a greater extent than with the effects of a direct receptor stimulant. The subjects were 256 male, Sprague-Dawley rats, 200-250 g. The measurement of stereotyped movements was performed in a Stoelting activity monitor, modified to permit quantification of restricted repetitive behaviors. This apparatus produced an analog signal with a frequency of oscillation equal to the frequency of occurrence of movements within the activity monitor. Twenty min. after an i.p. injection of either saline, phystostigmine, neostigmine, scopolamine, or methylscopolamine, subjects received a second i.p. injection of either saline, amphetamine, methylphenidate, or apomorphine. Amphetamine, methylphenidate, and apomorphine produced increases in the firing rate of hippocampal field CA1. The early appearance of opiate receptors is consistent with their being associated with either cholinergic or serotonergic neurons or both. Moreover, the early appearance of opiate receptors suggests that opiates or endogenous substances that act on opiate receptors might have effects not revealed by standard indices of opiate actions. Supported by NIMH Grant RSA10772.

1885 EFFECTS OF Picrotoxin and Physostigmine on Ethanol-Induced Inhibition of Hippocampal Unit Activity. Larry A. Grupp, Department of Pharmacology, University of Toronto, Addiction Research Foundation, Toronto, Canada.

We have previously demonstrated that high doses of ethanol inhibit spontaneous cell activity in the dorsal hippocampus of the awake rat. This report deals with the interaction of ethanol with each of two agents which increase the responsivity of hippocampal neurons by different mechanisms. Picrotoxin, a GABA antagonist, may, in part, produce an increase in neuronal excitability by dis inhibition, while physostigmine, an acetylcholine-esterase inhibitor, increases the excitability of cholinergic tone by maintaining high levels of acetylcholine. Rats chronically prepared with hippocampal microelectrodes, were pretreated intravenously either with picrotoxin (0.5 or 1.0 mg/kg) or physostigmine (0.15 mg/kg) 15 min prior to the infusion of ethanol (800 mg/kg). Recordings were taken for the entire pretreatment and ethanol periods (15 min each) and for a 15 min baseline period preceding the drug infusions. Picrotoxin pretreatment increased unit firing rate above baseline levels, produced a reduction in heart rate and induced epileptiform (spike and wave) activity in the frontal cortex. The subsequent administration of ethanol produced an inhibition of firing which did not differ in degree from that obtained without pretreatment, although the final firing level achieved was considerably higher than that in the absence of any pretreatment. Cortical EEG was briefly normalized to low amplitude fast activity before reverting back to spike and wave activity, and heart rate reduction appeared again higher than in the absence of pretreatment. Physostigmine pretreatment produced decreases increases in firing rate, yielded an essentially activated EEG, but did not decrease heart rate appreciably. The administration of ethanol for those units enhanced by the pretreatment brought about a quantitatively similar inhibition in rate to that obtained in the absence of any pretreatment although the firing level was again higher than in the absence of pretreatment. Those units inhibited by the pretreatment did not appear to show any further rate reduction following ethanol. EEG following ethanol showed little change from the activated mode, while heart rate also remained unaffected. Control experiments with neostigmine resulted in abrupt increases in rate, hemoglobin, and peripheral blood pressure. These data indicate that the manipulation of hippocampal responsiveness either by interfering with gaba-mediated inhibition or by augmenting cholinergically-mediated excitation does not alter the degree of firing depression produced by ethanol.


Initial studies of GABA-dopamine interactions in basal ganglia suggested that a striatogenic GABA system is inhibitory to the nigrostriatal dopamine pathway. However, direct injection into the ventral tegmental area or systemic administration of GABA agonists have produced facilitation rather than inhibition of motor behaviors thought to be mediated by the nigrostriatal dopamine system. As a part of a study of the role of GABA in an animal model of tardive dyskinesia, we made some further observations on this unexpected phenomenon by examining the effect of vinyl GABA, an irreversible catalytic inhibitor of GABA transaminase, on amphetamine stereotyped behavior. Eighteen hours after administration of vinyl GABA (1,000 mg/kg i.p.), control and treated animals (300 mg, male S-D rats) were given saline or dextroamphetamine (2 mg/kg). After two hours of behavioral observation, the animals were sacrificed and brains quickly removed and frozen for biochemical studies. In comparison with control animals, the vinyl GABA treated animals demonstrated an increase in the sniffing, gnawing stereotypy thought to be mediated through the mesostriatal dopamine system while locomotor activity mediated by mesolimbic striatum was totally abolished. Striatal homogenates showed a 2-fold increase in GABA, a 42% inhibition of GABA transaminase activity and a 27% reduction in succinic semialdehyde hydride activity. This dual action of elevated GABA on amphetamine stereotypy suggests differing interactions between GABA and dopamine systems in neostriatum and mesolimbic striatum. The results also provide additional evidence that treatments intended to increase GABA activity augment motor behavior mediated by neostriatum. Additional data, including results of GABA and dopamine binding to animals treated with neocuprine, will be presented.
NEUROPHARMACOLOGY

1887 ACTION OF CLONIDINE ON DOPAMINERGIC NEURONS OF THE SNAIL BRAIN
John C. Hancock, Department of Pharmacology, East Tennessee State University, College of Medicine, Johnson City, Tennessee 37601.

The action of clonidine (10^{-6} to 10^{-3}M) was studied on identified dopaminergic neurons in the abdabdomal ganglion of the snail, Helis aspersa. The electrode technique was used to establish presynaptic, postsynaptic and local anesthetic effects of clonidine. On cell RPal (Judge, S.E. et al. Comp. Biochem. Physiol., 70: 675-681, 1982.) clonidine caused depolarization of the cell membrane, a decrease in input resistance and an inhibition of spontaneous firing. The membrane response to clonidine was less than that caused by dopamine (10^{-4} to 10^{-3}M) or methoxamine (10^{-7} to 10^{-5}M) but greater than that caused by isoproterenol (10^{-6} to 10^{-3}M). Disruption of synaptic transmission by elevating extracellular dopamine concentrations did not alter the responses to these drugs. Dihydroergotamine (DHE: 10^{-6} to 10^{-3}M) but not chlorpromazine (10^{-6}M) or haloperidol (10^{-5}M) blocked the responses to clonidine, dopamine, methoxamine and isoproterenol on the post synaptic membrane. Stimulating the left pallial nerve evoked an inhibitory potential of long duration preceded by an EPSP (ILD-E) in cell RPal. Stimulating the anal nerve evoked an antidromic potential followed by multiple firing. DHE but not chlorpromazine blocked all sympathetically evoked potentials without affecting the antidromic potential. For threshold stimulation, complete block of the ILD required 31 ± 1.8 min. For supramaximal stimuli, blockage of the ILD required 31 ± 1.7 min while blockage of the ILD-associated EPSP required 1.5 ± 1.5 min. Blockage of multiple firing accompanied stimulation of the anal nerve required 61 ± 1.9 min. Following complete block of the synaptic potentials by clonidine, the cell membrane was normally responsive to dopamine (10^{-7} to 10^{-3}M).

The results in elevated Mg^{2+} demonstrate that clonidine has a direct post synaptic dopaminergic effect on the cell body of RPal and produces a dopamine-like response. The blockage of the responses to clonidine, dopamine, methoxamine and isoproterenol by DHE suggests that all of the drugs act on a dopamine receptor mediating inhibition. The failure to affect the antidromic potential eliminates the possibility of a local anesthetic action. The effect of clonidine to block synaptic potentials without affecting the cell response to dopamine suggests that clonidine has a presynaptic action to inhibit dopamine release.

1888 Desmethylamphetamine Increases Striatal Single Unit Activity in Freely Moving Rats. Eric L. Hansen* and James G. McElligott (Spon: E. Geller) Department of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Pa. 19140.

In awake freely moving rats, desmethylamphetamine (5 mg/kg, i.p.) significantly increased (p < .001) the firing rate of a group of individual striatal neurons (n = 13) by 100% during periods of drug-induced locomotion and stereotyped behavior. Individual cells increased their firing rate for periods ranging from 1 to 4 hours. However, the group as a whole had significantly elevated firing rates over the entire four hour time when contrasted with the preinjection rate. A group of saline (i.p. injection) control cells (n = 5) manifested no change in rate over a similar comparison period. This result agrees with a previous multi-unit recording study in freely moving rats which found that desmethylamphetamine (1-10 mg/kg, i.p.) caused small populations of striatal neurons to increase their average rate of discharge. In contrast, desmethylamphetamine has been reported to cause increases, decreases, or bi- and tri-phasic changes in firing of individual striatal neurons in immobilized, artificially resired rats. The discrepancy between data derived from freely moving and immobilized animals emphasizes the hazards of inferring neuronal correlates of desmethylamphetamine-induced behavior from experiments on immobilized non-behaving animals.

1889 BENZODIAZEPINE RECEPTORS IN PRIMARY CULTURES OF CEREBRAL CORTEX.
D.E. Harris and William F. Hood. V.A. Med. Center and Dept. Pharmacol. Univ. of North Carolina, School of Medicine, Chapel Hill, N.C. 27514.

Benzodiazepine receptors were studied in primary cultures of rat cerebral cortex using [1^H]-flunitrazepam (H-FNT) as a radioligand. Cultures containing mixed populations of neurons and glia were prepared from 18 day gestation rats and grown for 4 days. Scatchard analysis to membrane preparations obtained from these cultures revealed a single high affinity binding site with a K_d (~1.4 nM) identical to that observed in membrane preparations of adult cerebral cortex. Clonazepam, diazepam, oxazepam and chloraloside inhibited the binding of [1^H]-FNT with apparent K_i values (0.3, 0.8, 20, and 800 nM, respectively) that were similar to that observed for adult cerebral cortex. A variety of other non-benzodiazepine psychoactive drugs had little or no effect on binding at concentrations of competing drug up to 100 uM. The number of [1^H]-FNT binding sites, the greatest amount of [1^H]-FNT binding (101 ± 6 fmol/dish) and the highest specific activity of receptors (230 fmol/mg protein) increased the duration of the culture period resulted in a decrease in the number of binding sites per dish (day 8 = 44 ± 4 fmol/day; day 11 = 27 ± 4 fmol; day 15 = 13 ± 2 fmol). The decrease in [1^H]-FNT binding sites coincided with a similar diminution in the number of neuronal elements as determined by (1) phase contrast microscopy, (2) transmission and scanning electron microscopy, and (3) serotonin-stimulated Na^+ uptake. In contrast, the number of glial cells and amount of protein per culture increased markedly over the same time period. When neuron-containing cell clusters in 3 day cultures were separated from the neural cell bed by physical agitation, greater than 90% of the [1^H]-FNT binding sites were recovered in the cell cluster fraction. Growth of cultures in the presence of antimitotics resulted in virtual elimination of glial cells from the cultures with a much smaller reduction in the number of neurons. Under these conditions, the number of [1^H]-FNT binding sites were eluted from neurons and not glial cells. These data indicated that primary cultures of rat cerebral cortex may serve as a useful model system for the study of benzodiazepine receptors. Preliminary results suggest that specific [1^H]-FNT binding sites in these cultures may result primarily on neuronal elements.


Effects of ethanol and related drugs on synaptosomal calcium transport were investigated using the K^+ depolarization-stimulated uptake of 45Ca by intact synaptosomes (Blauwstein, J. Physiol. 247:617, 1975) and the ATP-dependent sequestration of 45Ca by intra synaptosomal organelles (Blauwstein et al., J. Gen. Physiol. 72: 15, 1978) isolated from whole brain. In vitro addition of ethanol (EtOH), acetylethlyl (Acet) or pentobarbital (PB) inhibited calcium transport by both these processes in a dose-dependent fashion.

Drug  K^+  K^+  K^+  +ATP  +ATP  +ATP

<table>
<thead>
<tr>
<th></th>
<th>nmol Ca/mg protein/1 min.</th>
<th>nmol Ca/mg protein/5 min.</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>50 mM</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>200 mM</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>2.9</td>
</tr>
<tr>
<td>Acetylethylyl</td>
<td>1.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>0.5 mM</td>
<td>2.6</td>
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*Significantly different from control p<0.01

Results are from rats; similar effects were obtained in mice. The effects of these drugs were reversed when the membranes were centrifuged and resuspended in drug-free buffer. The effects were apparent as soon as 1 min after drug addition and persisted at least 60 min. Ethanol inhibited the depolarization-dependent uptake equally over a range of assay temperatures (0° to 37°) but inhibited the ATP-dependent uptake only at higher temperatures.

Mice ingesting an ethanol-containing (7%/v/v) liquid diet for seven days displayed altered synaptosomal calcium transport as compared to pair-fed controls. Depolarization-dependent and ATP-dependent transport were reduced by about 25% and 15%, respectively, by chronic alcohol exposure and the inhibitory effects of ethanol and pentobarbital added in vitro were reduced by chronic alcohol ingestion. Data from several strains of mice suggest that this effect is correlated with the development of tolerance to the behavioral effects of ethanol. In summary, the depolarization-dependent uptake was more responsive to alcohol than the ATP-dependent storage of calcium. These processes may be related, respectively, by chronic alcohol exposure and the inhibitory effects of ethanol and pentobarbital added in vitro.

Seventeen New Zealand rabbits (male, wt. 2.3-3.7 kg) were used in a study of the respiratory and cardiovascular effects of morphine. During ether anesthesia, animals were decerebrated at the level of the superior colliculus and the trachea, femoral artery and femoral vein were cannulated. Upon completion of surgery, ether was removed and a minimum of one hour was allowed to elapse prior to the administration of morphine. Respiratory frequency and blood pressure were monitored. Heart rate (HR) and mean arterial pressure (MAP) were derived from direct measurements of blood pressure. Maximum changes occurred within 15 minutes of the intravenous administration of morphine. Results were statistically analyzed over 30 minutes. In one group of animals, morphine was administered as a single, 6 mg/kg, injection. A second group received three, 2 mg/kg, injections, administered 30 minutes apart. Measurements before and thirty minutes after the single, 6 mg/kg, injection are shown in Table 1. Measurements before and thirty minutes after each of three, 2 mg/kg, injections are shown in Table 2. All values are MEAN±SE.

**TABLE 1.**

<table>
<thead>
<tr>
<th>Resp. Freq. (Breaths/Min)</th>
<th>MAP (mmHg)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>6 mg/kg</td>
<td>25 ± 10</td>
</tr>
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</table>

**TABLE 2.**

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<thead>
<tr>
<th>Resp. Freq. (Breaths/Min)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>6 mg/kg</td>
<td>25 ± 10</td>
</tr>
</tbody>
</table>

1982 CYCLIC AMP AND OTHER ADENINE NUCLEOTIDES INHIBIT CA++-DEPENDENT POTENTIALS IN SYMPATHETIC POSTGANGLIONIC NEURONS. B.K. Henning, and T.P. Shea. Dept. of Neuropharmacology, Hope National Medical Center, Duarte, CA 91010, USA.

Previous studies in this laboratory have demonstrated that α-adrenergic agonists reduce the input resistance of postganglionic sympathetic neurons of the rat. This results in a depression of three Ca++-dependent potentials: the shoulder on the repolarizing phase of the action potential (HAP), the hyperpolarizing afterpotential (HAP), and the Ca++ spike. Cyclic AMP has been implicated in both the mediation of α-adrenergic responses and in the control of Ca++ metabolism. Thus, we have examined the effect of several adenine nucleotides on Ca++-dependent potentials recorded intracellularly from the rat superior cervical ganglion (in vitro).

Cyclic AMP depressed the HAP by 14% (n=4) at 0.01 mM and by 25% (n=9) at 1.0 mM. Dibutyryl 8-bromo cyclic AMP (n=3) also decreased the HAP by about 25% at 1 mM. A number of other parameters were measured in the presence of 1 mM cyclic AMP. The duration of the HAP was reduced from 400 msec to about 250 msec. The action potential amplitude was only slightly reduced (1-3%), but its shoulder was significantly decreased. The input resistance was not affected. The Ca++ spike elicited in 1 mM TTX and 10 mM TEA was depressed by cyclic AMP, and this effect was not blocked by the α-antagonist, phentolamine, indicating that the effect of cyclic AMP was not due to release of endogenous catecholamines. Thus, we have examined the effect of several adenine nucleotides on Ca++-dependent potentials recorded intracellularly from the rat superior cervical ganglion (in vitro).

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1989


It has been shown (1) that L-tryptophan (L-T) 0.03, 0.1, 0.3, and 1.0 g/kg, in an effort to produce a dose-dependent trend toward depression of spontaneous locomotor activity (SLA) in mice. The effect was statistically significant at the two highest doses. After oral administration of the two combined, DL-tryptophan (DL-T) in 180 mice. The whole brain and brain stem, excluding the cerebellum, was dissected out over ice, after cervical dislocation. 5-HT was extracted using the butanol method and assayed fluorometrically. The results showed that brain 5-HT increases in a dose-dependent manner as shown in the following table after oral L-T administration. It can be seen that brain 5-HT level reached a plateau at 0.3 g/kg. At this dose L-T did not significantly reduce the SLA. At higher doses although brain 5-HT did not rise further, the SLA was more and more depressed. It is possible, based on this evidence alone that a non-serotonergic mechanism is involved in L-T effect on SLA. Further support for this contention comes from our results with DL-T and L-T: DL-T by itself changed neither the brain 5-HT level nor the SLA. However, when combined with L-T it competitively inhibited depression of SLA by L-T. Brain 5-HT levels were raised in a manner identical to that observed after L-T alone. In summary we have demonstrated, in mice, an uncoupling of the rise in brain 5-HT level from SLA depression after L-T both in terms of the dose required to evoke these changes and in terms of the production of one response (increased 5-HT level) without the other (depressed SLA) through the use of a competitive inhibitor (DL-T).

<table>
<thead>
<tr>
<th>Dose</th>
<th>Brain 5-HT</th>
<th>Dose</th>
<th>Brain 5-HT</th>
<th>Dose</th>
<th>Brain 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg</td>
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<td>g/kg</td>
<td>ug/g</td>
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<tr>
<td>water</td>
<td>.683 ± .022</td>
<td>L-T 0.03</td>
<td>.743 ± .025</td>
<td>L-T 0.10</td>
<td>.806 ± .043</td>
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<tr>
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<tr>
<td>.728 ± .032</td>
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<td>0.30</td>
<td>.865 ± .047</td>
<td>0.961 ± .030</td>
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</tr>
</tbody>
</table>

1987

LATERALIZED PERIORAL SENSITORMOTOR FIELD ACTIVATED BY INTRANIGRAL GABAERGIC DRUGS AND BY SYSTEMIC APOMORPHINE AFTER 6-OHDA NIGRECTOMY. J.P. Huston, B. Neff*, G. Papadopoulos* and H. Volz*.

Institute of Psychology III, University of Düsseldorf, Düsseldorf, FRG.

Unilateral injection of the GABA agonist muscimol (20 mg in 0.1 μg saline) into the substantia nigra of rats caused contralateral turning as well as an asymmetry in responsiveness to tactile stimulation. I.e., contralateral to the side of the injection touching of the lip, cheek or vibrissae elicited a reflex that consisted of a withdrawal of the lip, followed by a quick orientation towards and biting of the probe. Bilateral injection of the GABA antagonist picrotoxin (200 or 300 mg/kg) induced rotation in the direction ipsilateral to the injected hemisphere, and higher ipsilateral responsiveness.

No systemic injection of apomorphine clearly primes the biting reflex to tactile stimulation of the perioral area in intact rats and guinea pigs. The apomorphine-induced perioral biting reflex is blocked on the side ipsilateral to the substantia nigra lesioned by 8 μg of 6-OHDA. I.e., apomorphine primed the perioral biting reflex contralateral to the 6-OHDA lesioned substantia nigra during periods of contralateral turning. Injection of d-amphetamine in these animals induced ipsilateral turning; increased responsiveness to tactile stimulation on the side ipsilateral to the lesioned substantia nigra, but did not prim the perioral biting reflex. These experiments demonstrate that: (a) transient reversible asymmetries in sensorimotor responsiveness correlated with direction of spontaneous turning, and (b) a neuropharmacological basis of the perioral biting reflex, probably involving substantia nigra mediated dopaminergic-GABAergic systems.

1988


H-dipropylacetate (DPA) and 4-amino-3-oxoacetic acid (AOAA) produced dose related elevations of brain GABA as well as protection against maximal electroshock seizures (MES) 150 μA, 0.2 sec, 80 Hz) in rats. The ED50 for DPA in MES (200 mg/kg) caused a 15% increase in whole brain GABA. In contrast, AOAA, 20 mg/kg, increased whole brain GABA by 60% but only produced a 10% increase in whole brain GABA during periods of contralateral turning. Since DPA and AOAA had similar effects in the intact SN, 20 mg/kg GABA (over 3 fold). In view of the modest effect of DPA on brain GABA levels we were interested in determining whether the GABA increase produced by DPA was associated with nerve terminals. For this purpose we developed a surgical transection technique to unilaterally sever all connections between substantia nigra (SN) and the forebrain. This resulted in the complete destruction of GABAergic fibers afferent to substantia nigra (SN) leaving glial cells and neuronal perikarya intact. One week postoperatively, GABA measurement in the SN of the transected side was 10-20% of control. In the SN largely devoid of GABAergic nerve terminals, AOAA, 30 μg/kg, produced a marked (2-fold) increase in GABA content. In contrast DPA, 300 mg/kg, did not change the GABA content in the GABA-denervated SN. Since DPA and AOAA had similar effects in the intact SN (25% increase in GABA), it appears that the elevation of GABA produced by DPA is dependent upon the presence of GABAergic nerve terminals, whereas AOAA primarily elevates GABA in non-nerve terminal components.

As we have previously reported (Iadarola et al., Soc. for Neurosci. 44, 1978 and 1979, and Neurochem. in press) an analysis of GABA increases in several brain regions revealed that areas which were most affected by DPA were among those least affected by AOAA.

Taken together, our experiments suggest that the antidepressant efficacy of these drugs correlates with their ability to raise GABA in nerve terminals in specific brain regions and not with increases in GABA content. We have obtained additional support for this conclusion by examining the effects of γ-vinyl GABA (a specific and irreversible GABA transaminase inhibitor) GABA concentrations in various brain regions and in the GABA-denervated SN.
1900


Intermittent stimulation of dopamine receptors by dopamine agonists such as apomorphine may cause behavioral hypersensitivity. To test this possibility we administered the following drugs: a) unoperated rats received apomorphine, 1 mg/kg i.p. once weekly for four weeks; b) some also received daily doses of L-dopa 250 mg/kg + carbidopa 25 mg/kg p.o.; 2a) substantia nigra-lesioned rats received apomorphine i.p. once weekly; 2b) some substantia nigra-lesioned rats received daily doses of L-dopa + carbidopa.

The unoperated and the lesioned groups developed behavioral hypersensitivity manifest by a gradual increase in motor activity and stereotyped behavior during the four weeks. This occurred more rapidly in lesioned rats. The unoperated and lesioned rats receiving L-dopa exhibited significantly less motor activity and stereotyped behavior than those not receiving L-dopa.

We believe that the more continuous stimulation of dopamine receptors in rats treated with L-dopa prevented the development of behavioral hypersensitivity during intermittent apomorphine injection.

Adult male Wistar rats were fed chronically a liquid diet providing 5% of the calories as ethanol (4.9 g/kg ethanol daily) while pair-fed controls received the corresponding diet with alcohol replaced by an equinolaric concentration of sucrose. Rectal temperature, and voluntary alcohol or morphine intake was measured in several groups of rats at various times during chronic ethanol treatment. The fall in rectal temperature after a challenge dose of ethanol (3.0 g/kg) was significantly lower in the alcohol group than in controls, indicating tolerance to ethanol-induced hypothermia as a result of chronic ethanol treatment. They also displayed cross-tolerance to the hypothermic effect of morphine (15 and 30 mg/kg), whereas no cross-tolerance to the hyperthermic effect of morphine (5 mg/kg) was seen. Administration of morphine (30 mg/kg i.p.) for 3 days resulted in tolerance to morphine hypothermia and also cross-tolerance to ethanol-induced hypothermia.

In other experiments, guinea pigs were treated by subcutaneous implantation of morphine (4 x 75 mg) or placebo pellets for 3 days. Longitudinal muscle/myenteric plexus preparations obtained from the morphinized animals showed tolerance to the inhibitory effect of morphine on the electrically evoked contraction, as measured by parallel shifts in the log dose response curves. The extent of shift was approximately 1.8 log units. The same preparations were cross-tolerant to atropine, showing a shift of about 0.8 log units in the LDR curves. Similar results were found in preparations, obtained from naive guinea pigs, preincubated with morphine in vitro (4 x 10^{-6} M for 16 hrs) before testing.

These studies fit with our hypothesis that tolerance and cross-tolerance among drugs develop to drug effects rather than to drug per se. Therefore drugs sharing a common effect, even by different mechanisms, may show cross-tolerance for that effect.
1907


α-Dihydropicrotoxinin (DHP) binding to mammalian brain membranes was inhibited by small amounts of the 100,000 x g supernatant fraction obtained from the same homogenate, and also by some purines and pyrimidines. Furthermore, DHP binding was inhibited by pharmacological concentrations of benzodiazepines, both depressant and excitatory. [3H] DHP binding to fresh rat cerebral cortex membranes was measured by a centrifugation assay, giving a B of 2-3 µM and a B at 4 pmol/mg protein. Binding was highly enriched in the synaptosomal and light microsomal fractions; it was inhibited by biologically active picrotoxinin analogs, barbiturates (both depressant and excitatory), e.g. convulsants (Tieku et al. Mol. Pharm. 14, 38 [1978]; Life Sci. 22, 1843 [1978]; Neuropharm. 15, 319 [1975]), and by pyrethroid insecticides (Leeb-Lundberg, Napias and Olsen, in preparation). All of these drugs appear to have physiological effects involving Gamma-amino butyric acid (GABA) receptor-regulated Cl- channels. The high affinity binding sites for these drugs (some Kd values in nM range) suggest the possibility of an endogenous ligand in Brain. DHP binding was inhibited 50% by 50 µg of spermatic protein from rat cortex homogenate, assayed in 1 ml at 10 without preincubation, using 1 mg/ml membrane protein. [3H] DHP at 10 nM, 20 Ci/mmol, in 0.1M NaCl, ph 7.0. 50% inhibition of DHP binding was also obtained with 100 µg of protein, [3H] DHP at 10 nM, 29 Ci/mmol, in 0.1M NaCl, 20 mM HEPES, 1 mM CaCl2, ph 7.0. 50% inhibition of DHP binding was obtained with 100 µg of protein.

1908


Kainic acid (KA) selectively destroys certain types of neurons when injected systemically or in various rat brain regions. This action is hypothesized to occur through a synergistic interaction with endogenously released glutamic acid. New data are reported which are consistent with this proposal (Nadler, Life Sci. 24: 289, 1979). Because baclofen (BF) has been reported to reduce the release of glutamic acid via the voltage-sensitive Na+ channels (Nakanishi, J. Pharmacol. 56: 150, 1978) it was of interest to evaluate the possible interaction of BF with KA. The effects of gamma-aminobutyric lactone (GABA) and GABA mimetics were examined.

In the present experiments, KA (10 mg/kg i.v.) was administered to rats, which were then observed for 90 min afterwards by 'blind', experienced observers. Wet dog shakes became apparent approximately 20-25 min after injection. At 30-35 min, hyperactivity was noted and was usually followed several min later by clonic seizures usually involving the jaw and forelimb. KA also elicited a unique behavior, persistent scratching of the face with the hind legs. The ability of test drugs (i.p.) to antagonize its effects was evaluated by pretreating rats 30 min prior to i.v. KA.

BF (10-20 mg/kg) reduced the incidence of KA-induced clonic seizures. This effect contrasted strikingly with its inability to prevent metrazol- or electroshock-induced seizures. Similarly GBL (30, 100 or 300 mg/kg) selectively antagonized KA-induced seizures. Hyperactivity was reduced by the highest doses of BF (20 mg/kg) and GBL (300 mg/kg) tested. KA induced suppression of the thalamocortical EEG antagonized by low doses of BF (3 mg/kg and up) and by high doses of GBL (300 mg/kg). None of these treatments altered the incidence of wet dog shakes following KA. Neither BF nor GBL antagonized KA-induced seizures. Intrastriatally administered KA in rats reliably reduces striatal choline acetyltransferase levels, presumably by depleting striatal cholinergic neurons (30-60 mg/kg i.p., at 1 hr before and again 2, 5 and 8 hr after intrastriatal KA, 0.5 µg, 1 µl) totally failed to antagonize this monochemical effect of KA.

These results indicate that BF and GBL, which are not effective in traditional models of anticonvulsant activity, ameliorate the convulsant action of KA, as well as other induced monochemical effects. The inability of BF and GBL to reverse KA-induced wet dog shakes and striatal choline acetyltransferase depletion suggests that the effects of KA may be mediated via multiple mechanisms. In the present study, increased haloperidol-induced catalepsy at anticonvulsant doses of drug. Synthélabo-LERS, 31, Ave P. V. Couturier, F 92220 Bagneux, France.

The control by GABA neurons of noregastriatal dopamine (DA) utilizing neurons has been demonstrated using both biochemical and behavioral assays. The present studies were designed to investigate the possibility that GABA mimetics decrease striatal DA release, block apomorphine stereotypical behaviour and potentiate neuroleptic (e.g haloperidol)-induced catalepsy. These effects have the potential to be useful for antipsychotic effects. However, in view of recent observations that DA agonists have biphasic actions in these test systems, we have assessed the range and character of drug actions on the interaction of a wide dose-range of GABA agonists and antagonists on DA-mediated effects. For these studies catalepsy was measured utilizing the four test in male albino rats after a single injection of haloperidol, i.p. of haloperidol. Stereotypes were rated in male albino mice after 0.5 mg/kg, s.c. of apomorphine. Direct acting GABA mimetics (SL 76 002, muscainol) showed a clearcut biphasic action: haloperidol-induced catalepsy was antagonized and apomorphine stereotypes enhanced at low doses (12.5 mg/kg i.p. for SL 76 002, 0.25 mg/kg i.p. for muscainol, p = 0.01 vs reference compound alone in both cases) and markedly potentiated catalepsy and diminished stereotypes at high doses (100 mg/kg, i.p. for SL 76 002, 2.0 mg/kg i.p. for muscainol, p = 0.01 vs haloperidol alone). Agents having a putative GABA receptor antagonist) increased haloperidol-induced catalepsy at low (0.03 mg/kg, i.p.) doses and antagonized catalepsy at higher (0.05 mg/kg, i.p.) doses. Muscainol (1 µg/kg, i.p.) does not alter the catalepy aspect of the response, but enhanced the stereotypic activity of haloperidol. The present results demonstrate that changes of GABA receptors may be biphasic: changes in sensitivity or existence of extra-synaptic receptor or which respond to injected GABA agonists or antagonists but which are relatively insensitive to alterations in the synaptic concentrations of this neurotransmitter.

Intracerebroventricular (ICV) cannulae were implanted in 92 day old male NSR Sprague-Dawley derived rats. Following a three week recovery period subjects were returned to the home environment for one hour daily until behavior stabilized (after eight days). On subsequent days and 30 minutes after the ICV administration of either saline or dibutyryl cyclic 3',5'-guanosine monophosphate (dbcGMP) animals were observed for one hour. Behavioral observations reported consisted of five consecutive one minute sessions taken at the beginning and end of the assay. Behavior observed consisted of two types. Normal exploratory measures were made in which the following showed: anxiety related behaviors and dynamic activity (forward motility, vertical exploratory activity (VE), total exploratory time (TE), and habituation rate (HR). Atypical behavior could be described by: catatonia (C), wet dog shakes (WDS), convulsions (CON), and characteristic cholinergic effects. Dose range was 5 to 100 µg with all doses administered in 10 µl of saline with an n of 5 to 7 subjects per dose.

There are four observable dose responsive effects of dbcGMP on exploratory activity. At doses between 5 and 10 µg there is a 40% decrease in DA without any decrease in TE, indicating a specific effect on motor activity sparing the motivational component. Between 10 and 100 µg there is a biphasic dose response on these same measures ranging from depression at 10 µg, excitation at 100 µg, and suppression at 100 µg. Concomitant with the depressed phase is an effect seen with neuroleptics and certain anxiolytic agents: a two-fold increase in exploratory behavior with bicuculline (at 100 µg). This is a linear log dose response decrease in VE activity with complete suppression at 100 µg. Appearance of atypical behavior began at 100 µg and disappeared when repeated dosing was stopped. WDS and C are reversible but CON behavior is invariably irreversible leading to extensive brain damage and death. The multiplicity of effects of dbcGMP and its effectiveness at low doses seems to suggest a complex modulatory role for this substance in neural mechanisms.


Among neuroleptics, clozapine is unique because it fails to produce extrapyramidal side-effects and it has little demonstrable dopamine blocking activity. The clinical use of this drug in Europe has firmly established its use as a potent antipsychotic. Moreover, clozapine-induced agranulocytosis is a significant side-effect. It is premature to speculate on the clinical relevance of these observations. (NSF PCM 77-21639 and NIH GRS RR05140A2)


γ-Aminobutyric acid (GABA) appears to be an inhibitory neurotransmitter in both vertebrates and invertebrates. In addition, recent studies have indicated that GABA receptors can be present at or near this receptor site, a modulator(s) of GABA binding, which can be removed by the detergent Triton X-100, and which may play a role in the mechanism of action of the benzodiazepines. In the present study was undertaken to describe the pharmacological, biochemical and kinetic properties of the GABA receptor in a variety of species. In an attempt to better define the characteristics of this study, [3H]-GABA receptor binding was studied in membrane fractions prepared from vertebrate whole brain or invertebrate cephalopod ganglia using a previously published procedure (Jones and Snyder, Mol. Pharm. 13:442, 1977). Specific GABA receptor binding was defined as the amount of 3H-GABA displaced by unlabeled bicuculline (0.1 nM). In tissue not treated with Triton, a significant amount of bicuculline-displaceable 3H-GABA binding was detected in the brains of all 19 vertebrate species studied, with the hagfish, the lowest vertebrate, binding twice as much [3H]-GABA as the spiny dogfish, the next oldest species. All other vertebrates bound similar amounts of 3H-GABA, being one-third to one-fourth that observed in the hagfish. In contrast, after Triton treatment, the hagfish displayed the lowest amount of bicuculline-sensitive 3H-GABA binding and, under these conditions, the amount of binding observed increased in an evolutionary fashion. Kinetic analysis indicated that Triton treatment enhanced binding in all vertebrates, except the hagfish, by increasing the affinity of the receptor for GABA. The measurable bicuculline-sensitive GABA receptor binding was noted in all invertebrate studies, suggesting that the receptors in these species are relatively insensitive to this antagonist.

These results suggest that bicuculline-sensitive GABA receptors are present in the brains of all vertebrates and that during the course of evolution there developed a tritonal-sensitive substance (a) whose presence modifies the kinetic properties of this receptor site. This data, taken together with a previous report (Helsen et al., Brain Res. 141:342, 1978) indicating a lack of benzodiazepine receptor binding in the hagfish, is further circumstantial evidence that these drugs may interact with a Triton-sensitive modulator(s) at the GABA receptor. (Supported in part by USFSS grants NS-13803 and an RCRA US-00315 (6.J.E.).

9H-SPIROPERIDOL BINDING SITES IN HUMAN CAUDATE AND PREFRONTAL CORTEX. M.E. Maguire, A.C. Andorn*, and L.E. Weber. Department of Pharmacology and Psychiatry, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

The examination of 9H-spiroperidol (9HSP) binding in rat striatum has shown the existence of two binding sites with different pharmacologies (Andorn and Maguire, Soc. for Neurosci. IV:504, 1978). The high affinity site (Kp200 µM) exhibits a pharmacology that resembles the peripheral cyclic 3',5'-GMP site. The second site (Kp200 µM) appears pharmacologically to be an a-adrenergic receptor even though dopamine is presumably the transmitter. Our data to date suggest that properties of 9HSP binding and the associated pharmacology are similar in postmortem human caudate.

Examination of human prefrontal cortex suggests only a small amount (if any) of a high affinity site, while the density of a lower affinity binding site is comparable to that seen in the caudate. However, the pharmacology of this lower affinity site differs markedly from the pharmacology of either of the caudate sites. Although neuroleptic potency was similar, and apomorphine and serotonin were potent competitors (apomorphine > serotonin), dopamine and norepinephrine competed only at µM concentrations. Our postmortem cortical data is similar to data recorded by others in frontal cortex and not only suggests that the prefrontal cortical site is not the same site as those observed in the caudate but that it is primarily serotonergic.

We obtained postmortem tissue samples from a male schizophrenic in whom no tissue or fluid concentrations of psychotropic agents could be detected at autopsy. 9HSP binding studies on prefrontal cortex and caudate show marked changes in affinities for agonists and antagonists. The differences in binding make it difficult to determine if a change in affinity also exists in prefrontal cortex although there are no immediately apparent differences between normal or unmedicated schizophrenic patients. It is premature to speculate on the clinical relevance of these observations. (NSF PCM 77-24693 and NIH GRS RR05140G2)

In restraining a rat in a plastic cage, morphine-induced increases in core temperature (CT) whether morphine is given i.p. or intracerebrally (i.c.). Perhaps, the blockade of increased locomotor activity (LMA) is not the same as in the attenuated rise in CT. The purpose of the present experiments was to compare the time course of morphine-induced increases in CT and LMA in the rat following the drug's i.p. or i.c. administration.

Two measures of LMA were used. One was in a circular open field (OPF) and the other was confinement activity (CMA) which records activity when the rat rears on its hind legs. OPF counts were totaled at 15-min intervals following the injection of morphine in male rats, whereas CMA counts were totaled at 30-min intervals. CT was measured at 15 or 30 min intervals. Morphine was given i.p. in doses of 5, 15 and 30 mg/kg. It was given i.c. into the pre-optic anterior hypothalamus (POAH) in doses of 10, 20 and 50 µg. There was also a control group given the vehicle solution by each route.

In comparing the pattern of LMA and CT changes following i.p. morphine treatment, the time interval in which motor activity was greatest either preceded (2/6 treatments) or was coincident with the greatest recorded CT (4/6). If the OPF test, the peak level of activity was recorded 30-60 min before the peak CT after the 30 and 15 mg/kg doses of morphine, whereas the 5 mg/kg dose and all doses following each dose of morphine given i.c. Although an increase in CT was observed following the i.c. microinjection of morphine, the increase in CT preceded the increase in LMA activity in 5/6 treatment groups. On the other hand, the peak periods for LMA and CT were coincident in 3/6 treatment groups, while the peak LMA followed the peak CT in 2/6 treatments and preceded it 1/6.

These data indicate that a causal relationship may exist between the increase in LMA and the rise in CT observed following the i.p. administration of morphine. If true, this may partially explain the action of restraint on morphine-induced changes in CT. Since the increase in CT preceded the increases in LMA following the P0AH injections of morphine, LMA is probably not crucial to the initiation of this hyperthermic response, but may contribute to its prolongation.

1916 KETAMINE BINDS TO BRAIN OPIATE RECEPTORS. Lawrence H. Matts, Dopi A. Teljani, and Joseph R. Blanchine. Dept. of Pharmacology, College of Medicine, The Ohio State University, Columbus, OH 43210.

Ketamine hydrochloride (2-[(o-chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride) is a short-acting, anesthetic analgesic with unique side effects including ataxia, catalepsy, and epileptiform EEG's. The mechanism by which ketamine causes analgesia and its associative anesthesia is unclear. To determine whether an interaction with the endogenous opioid system might be part of the mechanism by which this drug acts, we have performed opioid binding assays to discover if ketamine displaces the stereospecific binding of [3H]-naloxone to the opiate receptor.

The assay system used is essentially the same as that described by Pert and Snyder (PNAS 70: 2243, 1973). Briefly, after killing a rat by decapitation, we excised the brain and removed the cerebellum from the brain. We then homogenized the brain in 50 mM Tris-HCl, pH 7.4. Two ml aliquots of the brain homogenate were incubated with various concentrations of drug in the presence of [3H]-naloxone (45,000 cpm) for 3 hrs at 0 C. Stereospecific binding was then measured by counting [3H]-naloxone in the presence of 1 µM dextrorphan minus its binding in the presence of 1 µM (-)-naloxone.

Ketamine was approximately as effective as morphine in displacing [3H]-naloxone from opiate receptors. Preliminary results suggest that 3/6 of both ketamine and morphine are about 10 nM. (This work was supported by gifts from Mrs. Marion Colwill and Mr. Max Weiss.)


Kainic acid causes a selective degeneration of neurons in the cochlear nucleus which receive primary auditory input. In this study kainic acid, 0.5 g in phosphate buffered saline was injected in the fourth ventricle adjacent to the cochlear nucleus of adult guinea pigs. In the short time periods, this dose of kainic acid causes a marked neuronal loss in the rostral anteroventral cochlear nucleus (24 and 17 days after injection, Gulley, 1979). Animals injected with kainic acid were placed in a sound-reducing auditory chamber for four hours. Previous studies in this acoustic environment have shown that auditory activity is sufficiently reduced to produce significant morphological changes in the presynaptic terminals after 24 hours (Gulley, Mattea, and Ulrich, personal observation). Animals received either injection of phosphate buffered saline followed by four hours of sound reduction or 0.5 g of kainic acid followed by four hours of exposure to ambient noise. Neuronal degeneration in the cochlear nucleus was observed in animals injected with kainic acid after both sound deprivation and exposure to ambient noise. The amount of neuronal degeneration was identical in both groups. No degeneration was observed in sound-reduced animals injected with vehicle buffer. Zaczek et al. (1978) described attenuation of the effect of kainic acid by various anaesthetics which limit neuronal excitation. Our data suggest that in the cochlear nucleus, decreased sound-evoked activity does not protect postsynaptic neurons from mildly toxic doses of kainic acid. This observation supports the findings in the cochlear nucleus that presynaptic terminals are not essential to mediate kainic neurotoxicity (24 and Gulley, '79).

1918 STUDIES ON PHENCYCLIDINE (PCP) LOCALIZATION IN RAT BRAIN. Richard C. Methc, Stanley P. Gluck, and Saul Magrill. Department of Pharmacology, Mount Sinai School of Medicine, New York, New York 10029.

The distribution of PCP within the brain was determined following injection of [3H]-PCP. Surprisingly, the distribution closely paralleled that of DOG changes, with the anterior cingulate having the greatest uptake.

These studies suggest the possibility that PCP may act primarily in the limbic system and could possibly account for the fact that PCP abuse is often associated with severe emotional disturbances.

Supported by NIH Grant NS 1-ROI-15058
1919

EFFECTS OF AMANTADINE DI RAT PLASMA PROLACTIN LEVELS.
H.Y. Meltzer and V.S. Fang, Depts. of Psychiatry and Medicine,
Univ. of Chicago Pritzker Sch. of Medicine, Chicago, Ill. 60637.

Amantadine (A) is an anti-parkinsonian drug whose mechanism of
drug action is uncertain. There is much evidence against
the suggestion that it acts via enhancement of dopamine
release or inhibition of DA uptake. It has also been
proposed (Cox and Tha, Eur. J. Pharmacol., 30, 34, 1975) that
A may increase receptor sensitivity to serotonin (5-HT).

We have investigated these hypotheses by studying the ability of
A to influence the increase in rat prolactin (PRL) secretion
induced by haloperidol (H) a dopamine antagonist, and 5-hydroxy-
tryptophan (5-HTP), the precursor of 5-HT. We have previously
shown that drugs which release DA and/or block its reuptake
such as d-amphetamine and methylphenidate antagonize the H-
and 5-HTP-induced increases in plasma PRL. A in doses of 50
and 100 mg/kg ip markedly enhanced the increase in plasma PRL
produced by 5-HTP 100 mg/kg ip (168 SE 18 ng/ml; 168±36 ng/ml
and 78±21 ng/ml respectively). On the other hand, A 50, 100
and 150 mg/kg did not potentiate the increase in PRL produced
by H. These results suggest A does enhance 5-HTP activity
and does not enhance DA release or block its reuptake. Studies
concerned with a specific increase in 5-HT receptor sensitivity
following treatment with A will be reported.

1920

SUPERSENSITIVITY AND INCREASED NUMBER OF OPIATE RECEPTORS IN
CEREBRAL HEMISPHERES OF RATS WITH HEREDITARY DIABETES INSIPIDUS.
Rita B. Messing, Beatriz J. Vasquez, Henk Rigter*, Robert A. Jenser
Joe L. Martinez, Jr., J.C. Crabbe, Jr.*, and James L. McGaugh.
Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717.

This study investigated opiate receptors in rats with heredi-
tary hypothalamic diabetes insipidus (Brattleboro strain). These
raths have no detectable brain or neurohypophysial vasopressin.
They also have deficiencies in brain peptides, pituitary hormone,
tolerance development to opiates. Therefore, "H-dihydroxyphorine
(DM) binding kinetics were examined in 6 brain areas of rats
homozygous for the mutant autonomic gene (DHM) and hetero-
sipids (HO-DI rats). DM binding of these rats was compared to
binding in rats heterozygous for diabetes insipidus (HE-DI rats)
and normal rats. Washed membranes were pre-incubated at 37°C
in 0.05M tris HC1 buffer, pH 7.4 with 100 nM of either dextromorph
or levorphanol. Samples were then incubated with one of 7 con-
centrations of "H-DM ranging from 0.2 to 13 nM, and stereospecific
binding was determined at each concentration. Apparent associa-
tion constants (K1's) and receptor concentrations (Bm a x ')s for
each brain region were obtained from Scatchard plots of the data.

<table>
<thead>
<tr>
<th>BRAIN REGION</th>
<th>APPARENT K1(Bq/mg protein)</th>
<th>Bmax(fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant. Cortex</td>
<td>3.77</td>
<td>1.54*</td>
</tr>
<tr>
<td>Normal</td>
<td>6.17*</td>
<td>80.9</td>
</tr>
<tr>
<td>HO-DI</td>
<td>4.56</td>
<td>127.2</td>
</tr>
<tr>
<td>Striatum</td>
<td>6.01</td>
<td>179.3</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>7.88</td>
<td>20.9</td>
</tr>
<tr>
<td>Thalamus</td>
<td>5.47</td>
<td>50.0</td>
</tr>
<tr>
<td>Midbrain</td>
<td>7.31</td>
<td>375.2</td>
</tr>
</tbody>
</table>

* and **: p<0.02, p<0.01 respectively, different from normal.

Higher receptor concentrations and affinities for DM were
observed in all assayed areas of the cerebrum (anterior cortex,
amygdala and striatum) to normal rats (see table). In contrast, in assayed areas of the brainstem
afferent or number were found between HO-DI and normal rats (see table). In general, DM binding kinetics in cerebral hemi-
spheres of HE-DI rats were intermediate to those of HO-DI and
normal rats, but HE-DI rats had significantly fewer opiate recep-
tors in the thalamus and midbrain when compared to normal rats.

Our findings suggest that opiate receptor differences between
HO-DI and normal rats may be involved in impaired tolerance develop-
ment of Brattleboro USPHS AGG008 rats.

1921

HALOTHANE EFFECTS ON SENSORY EVOKED PHOTIC RESPONSES RECORDED
SIMULTANEOUSLY FROM RETICULAR FORMATION, THALAMUS AND CORTEX
IN FREELY MOVING ANIMALS. Luis J. Moreno* (SPOM,W,S,Fielde)
The University of Texas Medical School at Houston, Houston,
TX 77025.

Sensory evoked potentials were recorded simultaneously in
response to sensory stimulation from three areas within the
central nervous system which have been postulated to be in-
volved in mechanisms of general anesthesia. Permanent
semimicroelectrodes (62 µm in diameter) were implanted
stereotaxically under pentobarbital (50 mg/µg) anesthesia in
the brainstem reticular formation (MRF), ventral posterior
cortex of the thalamus (VPL) and the somatosensory cortex
(SMA) of rats whose nigral cell discharge rate had been reduced
little or no effect on spontaneous firing rate. In
in vivo experiments, rats were anesthe-
mized with chloral hydrate. Single dopamine-contain-
ing neurons in the pars compacts of the substantia
nigra were identified on-line by their firing rate
(2-7 spikes/sec) and spike duration (> 2 msec)
characteristics. EN285 (up to 0.4 mg/kg, i.v.) had
little or no effect on spontaneous firing rate.

Rats whose nigral cell discharge rate had been reduced
40-60% by d-amphetamine pretreatment, EN285 (0.1-0.4
mg/kg, i.v.) reversed the amphetamine-induced depres-
sion in a dose-dependent fashion. The present work
suggests that EN285 has central dopamine antago-
nist properties.

1922

DOPIAMINE ANTAGONIST ACTIVITY OF A NOVEL ANTI-EPSYCHOTIC
COMPOUND EN285. Paul E. Myers, Greg R. Christoph, and
Rebecca S. Knight*. Central Research & Development
Department, E. I. du Pont de Nemours & Co., Inc.,
Wilmington, DE 19898.

Neurochemical and electrophysiological methods were
used to study the dopamine antagonist properties of
EN285, a chemically novel methanocyclopyrrole.
EN285 was an effective antagonist of rat striatal dopamine-sensitive adenylate cyclase with an IC50 6 x 10-7 M; dopamine 1 x 10-5 M.
The maximal stimulation of adenylate cyclase activity as
measured by cAMP production was observed at 6 x 10-7 M
dopamine with maximal stimulation at 1 x 10-5 M dopamine.
Trifluoperazine showed 50% inhibition at 1 x 10-7 M.

The potency of EN285 is similar to that of
chlorpromazine (IC50 6 x 10-7 M; Clement-Corail et al.,

In electrophysiological experiments, rats were anesthe-
tized with chloral hydrate. Single dopamine-contain-
ing neurons in the pars compacta of the substantia
nigra were identified on-line by their firing rate
(2-7 spikes/sec) and spike duration (> 2 sec)
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Rats whose nigral cell discharge rate had been reduced
40-60% by d-amphetamine pretreatment, EN285 (0.1-0.4
mg/kg, i.v.) reversed the amphetamine-induced depres-
sion in a dose-dependent fashion. The present work
suggests that EN285 has central dopamine antago-
nist properties.

Since ethanol is the oldest known "anxiolytic" agent, and since anxiolytic benzodiazepines and barbiturates are known to potentiate GABAergic neurotransmission, the following investigations were asked: 1) Does ethanol potentiate the inhibitory effect of GABA on single neurons in the feline cerebral cortex? 2) If there is such a potentiation, is it specifically exerted on inhibitions evoked by GABA or does it involve inhibitions evoked by other postulated neurotransmitters, such as glycine, serotonin and dopamine? The experiments were performed using the "isolated cerebrum" unanaesthetized preparation. The neurons were recorded with standard microelectrode techniques. The degree of inhibition of single cortical units induced to fire submaximally by Na-L-glutamate was measured from peri stimulus histograms generated by the extracellularly recorded spikes.

It was found that ethanol released from microperitts (0.3 M in 165 mM NaCl) by "electro-osmosis" (by removing the retaining current or by ejecting currents up to 10 mA) or applied intravenously (0.2-2.0 mg/kg) potentiated strongly the inhibition of neuronal firing produced by iontophoretically-applied pulses of GABA. If it had an effect on inhibitions evoked by pulses of glycine, and had an antagonistic effect on the inhibition produced by pulses of serotonin or dopamine. Furthermore, ethanol and by "electro-osmosis" in the presence of nipecotic acid, and to be recognized by other mechanisms, but not by endogenous GABA. In the aforementioned electro-osmotic and intravenous doses frequently decreased spontaneous firing as well as the firing evoked by Na-L-glutamate. Several minute hourly post application of doses higher than those affecting the inhibitions under study and precautions were always taken to use doses of ethanol that did not interfere with Na-L-glutamate. When tested on the same neurons, the effects of ethanol on GABA pulses and electrophysiologically-evoked cortical inhibition were identical with the effects of fluropentazol and chloralhydrate, except that the effects of benzodiazepines lasted longer.

The above findings may have implications for the etiology and treatment of alcoholism. Supported by the Medical Research Council of Canada.


Ethanol (3g/kg) was found to increase the period of recurrent inhibition recorded in hippocampal neurons in response to commissural fiber activation. This increase in the length of inhibition may be due to increased excitability of pyramidal neurons in the presence of ethanol.

Single unit responses were measured in CA1 and CA3 areas of the dorsal hippocampus using 3M NaCl-filled glass microelectrodes. Online computer-generated post-stimulus-time histograms provided a measure of the degree of inhibition of spontaneous firing following stimulation. Stimulating current was delivered through bipolar stainless steel electrode positioned in the contralateral hippocampus. Pyramidal neurons, as identified by firing pattern, responded to commissural fiber stimulation with an initial excitatory response followed by a cessation of activity lasting from 10-300 msec. As previously reported (Spencer and Kendel, Exp. Neurol. 1961) increasing intensities of stimulus voltage produced longer periods of recurrent inhibition. However, after a single i.p. injection of ethanol (3g/kg), the stimulus response curve for the period of recurrent inhibition declined over the up to 10 min interval, indicating an increase in the time course for inhibition with a given stimulus current. This increase in the time of recurrent inhibition could be due to several mechanisms, including: 1) an increased activation of pyramidal neurons which then activate more inhibitory interneurons; 2) an increased activation of inhibitory interneurons; and 3) an increased transmitter release by inhibitory interneurons in the absence of increased activation of pyramidal neuron activity; 3) a change in pyramidal cell responsiveness to inhibitory synaptic input.

Our findings that iontophoretic application of ethanol (1-3M) on to CA1 and CA3 hippocampal neurons increased spontaneous firing rate in the majority of cells tested would be consistent with a decrease of increased pyramidal neuron excitability after ethanol. Moreover, an analysis of field potentials evoked by commissural stimulation revealed that the size of the population EPSP did not change while the size of the population spike was affected in the presence of ethanol.


Animal responses to several different types of opiate analgesics have suggested multiple classes of receptors (Martin et al., JPET 197 57, 1976). The present studies were performed to determine whether specific opiate receptors of high specific affinity and specificity for a single class of receptor.

When tested on the same neurons, the effects of ethanol on GABA pulses and electrically-evoked cortical inhibition were identical with the effects of fluropentazol and chloralhydrate, except that the effects of benzodiazepines lasted longer.

The above findings may have implications for the etiology and treatment of alcoholism. Supported by the Medical Research Council of Canada.


The rate of dopamine formation in rat brain striatal synaptosomes markedly increases as the pH is lowered from 7.2 to 6.2. Compared to pH 7.2, synthesis was increased 80% at pH 6.6 and 10% at pH 6.2. However, at pH 6.2, synthesis is accompanied by a significant increase in apparent Km for tyrosine as well as in apparent Vmax. Although these kinetic changes are similar to that introduced by the depolarizing agent veratridine, it does not appear that synthesis is stimulated at pH 6.2 via synaptosomal depolarization since (1) synthesis stimulation still occurs at pH 6.2 in a calculation of the percentage of stimulation observed with veratridine (2) tyrosine uptake is not inhibited at pH 6.2, in contrast to the inhibition of tyrosine uptake produced by veratridine.

In order to study how the regulatory properties of synaptosomal preparations may vary according to pH, we have examined the effects of various stimulatory and inhibitory agents at pH 7.2, 6.6 and 6.2. The effects of three stimulatory agents (veratridine, amphetamine and phenylethylamine) were significantly diminished at pH 6.2 compared to pH 7.2. Their effects at pH 6.6, however, were quite similar to those at pH 7.2. The effects of two inhibitory agents (dopamine and tyramine) were also significantly antagonized at pH 6.2 compared to 7.2, while their effects at pH 6.6 were similar to those at pH 7.2. These results suggest that caution must be exercised in choosing an appropriate "optimal" pH for studying synaptosomal synthesis regulation, but it is possible to increase activity by altering pH without necessarily altering the regulatory properties of the system.
1927 A GENERAL METHOD FOR AUTORADIOGRAPHIC VISUALIZATION OF BRAIN RECEPTORS IN VITRO. Candace B. Pert and Miles Herkenham. Biological Psychiatry Branch and Laboratory of Neurophysiology, NIMH, Bethesda, MD 20025

Previous autoradiographic visualization studies have involved either the injection of reversible radioactive ligands into the tissue with subsequent work-ups developed for diffusible substances ("in vivo") or incubations "in vitro" with ligands which form covalent bonds. Since highly specific ligands form covalent bonds with receptors are not always available, we developed a method for in vitro labeling of receptors which features a fixation procedure of general applicability.

Rats were decapitated, their brains were rapidly removed, frozen on dry ice, cut into 25 micron sections in a cryostat (-12°C), pressed lightly onto slides, and maintained at 4°C for several days. Batches of slides were incubated at 4°C in 200 µl of sodium phosphate buffer (0.05 M, pH 7.4) containing bacitracin (500 µg/ml), aprotime (1 µg/ml), 100 µM sodium chloride and [3H]dipropomiphene (1 x 10^6 M) for 10 min. Slides were quickly dipped into five serial washes of 0.2% albumin at 0°C and a final wash of distilled water at 0°C and rapidly frozen with dry ice. After lyophilisation overnight, sections were fixed by overnight exposure to concentrated glutaraldehyde vapors in a sealed dish. Finally, the slides were washed in a series of five 10-min rinses in 1 M glycine (pH adjusted to 7.4) and two washes in distilled water at room temperature. The clean dried slides were dipped in the darkroom into Kodak NTB-2 emulsion (1:1 dilution) and processed by traditional autoradiographic methods.

Slides available at 2 weeks of exposure showed tissue preservation consistently comparable to conventionally fixed tissue, very low background grain counts, comparable low levels in white matter and striking clusters of grains overlaying several brain areas previously determined to be enriched in opiate receptors, e.g., striatal and hippocampal EEG. EEG, heart rate, temperature and postural EEG synchrony during a non-sleeping behavior dissociation (EEG synchrony during a non-sleeping behavior) was evident after 33 µg/kg and after 33 µg/kg of clonidine (11, 33 and 100 µg/kg) on the EEG and behavior of the intact unrestrained beagle dog prepared with chronic electrodes for recording cortical and hippocampal EEG. EEG, heart rate, temperature and respiratory rate were recorded before and after injection with 20 mg/kg of lidocaine. The dogs were in a modified chamber and their behavior was observed on a video monitor. Drugs were injected intravenously over 10 min from a pump outside the chamber. Morphine and clonidine produced similar EEG and behavioral changes for which there was greater evidence in parietal than occipital recordings. EEG, behavorial dissociation (EEG synchrony during a non-sleeping posture) was evident after all doses of morphine and after 33 µg/kg and 100 µg/kg of clonidine. Also, both drugs increased total sleep. The increase was dose related after morphine, whereas the sleep after clonidine was maximal after the 33 µg/kg dose.

Spectral analysis of the EEG in clonidine treated dogs indicated that the effect of the drug was maximal at 15 min. The cut off frequency (half-power point) was increased from 6 to 10 Hz in the parietal and occipital EEG by clonidine. The drug increased power in the 4-8 Hz region while it decreased power in the 0.5-4 Hz band. The later effect was evidenced by lightening of the EEG and behavioral effects of morphine (1 mg/kg) but not those of clonidine (33 µg/kg). These data are consistent with the proposal that morphine and clonidine act upon different receptors in neural pathways which mediate these EEG and behavioral effects.

1929 MORPHINE-LIKE EFFECTS OF CLONIDINE ON THE EEG AND BEHAVIOR IN THE DOG. Wallace B. Pickworth, Lawrence G. Sharpe and Vaikunth N. Gupta*. National Institute on Drug Abuse, Addiction Research Center, Lexington, Kentucky 40503

Clonidine, a non-opioid agonist, has several effects similar to those of the opiates. We have compared the effects of morphine (0.5, 1.0 and 2.0 mg/kg) and clonidine (11, 33 and 100 µg/kg) on the EEG and behavior of the intact unrestrained beagle dog prepared with chronic electrodes for recording cortical and hippocampal EEG. EEG, heart rate, temperature and respiratory rates were recorded during a 2 hr experiment where each dog was in a modified chamber and their behavior was observed on a video monitor. Drugs were injected intravenously over 10 min from a pump outside the chamber. Morphine and clonidine produced similar EEG and behavioral changes for which there was greater evidence in parietal than occipital recordings. EEG, behavorial dissociation (EEG synchrony during a non-sleeping posture) was evident after all doses of morphine and after 33 µg/kg and 100 µg/kg of clonidine. Also, both drugs increased total sleep. The increase was dose related after morphine, whereas the sleep after clonidine was maximal after the 33 µg/kg dose.

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1929 D-AMPHETAMINE REDUCES STRIATAL SUBSTANCE P CONCENTRATION BY PRESYNAPTIC RELEASE OF DOPAMINE. D. J. Pettibone and R. J. Wurtman. Dept. of Nutrition and Food Science, M.I.T., Cambridge, MA 02139

We have previously shown that the acute administration of d-amphetamine, a drug which releases dopamine from neurons, significantly increases the concentration of substance P in the rat striatum (Pettibone, Wurtman and Leeman, Biochem. Pharmacol., 39:839, 1978). We suggested that the reduction might be mediated by activation of dopaminergic receptors. This report further characterises the effects of amphetamine on striatal substance P and shows its dependence on adequate striatal dopamine concentrations.

The intravenous administration of d-amphetamine sulfate to male, Sprague-Dawley rats (175 g) causes a dose-dependent reduction in substance P immunoreactivity within the striatum; a 10mg/kg dose reduces substance P immunoreactivity by 2.37 to 0.97 pmoles/mg tissue (p<0.01), 6 hrs post-injection. Substance P levels of substantia nigra or hypothalamus are unaffected. The amphetamine-induced reduction in striatal substance P is blocked in animals pretreated with haloperidol (1.3, or 10mg/kg i.p.). Similarly, when striatal dopamine levels are reduced 70-75%, either by injections of α-methyl tyrosine methyl ester (25mg/kg i.p.) or by lesions of the nigrostriatal tract (induced by intracerebral injections of 6-OHDA), the effect of amphetamine is prevented.

These data indicate that the mechanism by which d-amphetamine reduces striatal substance P content involves the presynaptic release of dopamine from the terminals of nigrostriatal neurons. Together these observations provide the possibility that striatal dopamine release may normally participate in the regulation of neuronal substance P concentrations in this brain region. (Supported in part by NIMH grant AM-4228)

1929 POSSIBLE DISRUPTION OF GABAergic NEURAL FUNCTION IN ADULT MICE TREATED AS NEONATES WITH MONOSODIUM GLUTAMATE. William J. Pizzi, James R. Unmestaw* and June E. Barnhart. Department of Psychology, Northeastern Illinois University, Chicago, IL 60625

Mice, treated as neonates with monosodium L-glutamate(GLU), exhibit a sequelae of somatic and behavioral deficits as adults due to the neurotoxic effects of GLU on the developing nervous system. We have recently shown that neonatal GLU-treated mice are more susceptible to pentylenetetrazol (PTZ) induced convulsions as adults. In order to explore this phenomenon, we have compared the differential response of GLU-treated and control animals to convulsant phencyclidine (PCP) and to convulsants produced via differing pharmacological mechanisms.

Subjects were male HA:ICR mice born in our laboratory and housed with their dams until weaning at 29 days of age. Experimental animals were injected for 10 consecutive days (days 2-11 postpartum) with a gradually increasing dose of monosodium L-glutamate (2.2-4.4 mg/kg body weight). Control subjects received equivalent volumes of equimolar NaCl. All animals were treated between 90-120 days of age. The convulsant potencies of 3-mercaptopropionoate (3-MP), an inhibitor of gamma-aminobutyric acid (GABA) synthesis, and bicuculline-HCl (BIC), a GABA receptor blocker, were determined according to the method of Litchfield & Wilcoxon (1949) using the least-squares criterion. A positive response was defined as a generalized clonic convulsion with loss of righting reflex. All agents were administered intraperitoneally using a 500 µl syringe.

GLU-treated mice were significantly more susceptible to 3-MP induced convulsions than control animals. PCP (1mg/kg, i.p.) was also more effective in inducing convulsions in GLU-treated animals (p<0.05). The chemical convulsant 3-MP induced a 70-75% reduction of striatal dopamine levels in GLU-treated animals (p<0.05). The reduction in striatal dopamine levels was not prevented by the administration of bicuculline-HCl (2mg/kg, i.p.). The findings that GLU-treated animals are more sensitive to 3-MP and that they show a decreased sensitivity to BIC-induced convulsions suggest a potential role for GABAergic neurotransmission in these animals. It is possible that GLU, administered during a critical period of neurochemical development, may permanently interfere in GABA synthesis and metabolism. Decreased GABA production may lead to altered sensitivity or increased numbers of GABA receptors, thus explaining the decreased sensitivity of GLU-treated animals to BIC.

1930 NEUROPHARMACOLOGY
1931 REGIONAL LOCALIZATION OF D-AMPHETAMINE IN RABBIT BRAIN AFTER INTRACEREBROVENTRICULAR ADMINISTRATION: EFFECT OF NALOXONE

Albino rabbits were anesthetized (Ketamine, 55 mg/kg + chlorpromazine, 5 mg/kg) with a cannula implanted into the lateral ventricle. Seven days after surgery, a 50 μl Merles solution containing 96 μg 1-14C-2-14C-methadone was introduced into cerebral ventricular system. The dose exerted no noticeable behavioral effects. Following decapitation at 0.25, 0.5 or 2 h, several brain areas were assayed for 14C-methadone. 14C was unevenly distributed in various regions reaching peak levels within 15 min. The superior colliculus, tegmentum, pons, hypothalamus, caudate, inferior colliculus, septum-nucleus accumbens, and medulla contained from 1.3 to 5.8 µg/g while olfactory tubercle, cortex, cerebellum, hippocampus and superior colliculus contained less than 1.0 µg/g. 14C-methadone considerably declined at 2 h. Pretreatment with naloxone (10 mg/kg i.p., 15 min) produced no significant effects on methadone levels in several brain regions examined at 15 min after methadone administration; only statisitically significant differences were: marked decreases in inferior colliculus, superior colliculus and hypothalamus, a samll decrease in the thalamus, a large increase in the caudate and a small increase in medulla. (Supported in part by the Ensor Research Foundation and V.A. Institute Funds Project No. 7155-03).

1932 THE EXCITATORY PORTION OF THE BIPHASIC RESPONSE OF CENTRAL NEURONS TO LIBUTERIC ACID. E. Pull, Deps. of Anesthesia and Pharmacology, University of British Columbia, Vancouver, B.C., Canada, V7W 1W5.

At the last meeting of this Society, Pull and Kmjevic reported (Neurosci. Abstr. 4:431, 1978) that microiontophoretic applications of ibotenate, a D-amino acid analog of L-glutamic acid, characteristically produced a biphasic effect in cerebral cortical neurons, as in spinal neurons: a strong excitation followed by a powerful inhibition of L-glutamate-evoked discharge. A similar effect was seen with ibotenate-evoked discharge. As in the case of the event-related potentials of neurons to L-glutamate by apomorphine, the depolarization-evoked firing was dose-related and at subthreshold amounts of ibotenate could be prevented by concurrent, extracellular application of methohexitol.

Since the long latency of onset and afterdischarge of the excitatory responses of cortical neurons to ibotenate appeared to be more prominent in cats anesthetized with diallylbarbiturate than with methoxyflurane, the effects of DL-ibotenate in cerebral cortical neurons were examined both extra- and intracellularly using parallel microcathode assemblies (triple barrel glued to single barrel at intertip distances of 780 μm) in cats with their forebrain isolated by leucotomy during brief anesthesia with halothane. The most typical response of pre- and post-cruciate cortical neurons to iontophoresis of DL-ibotenate in nonanesthetized preparation, was a strong excitation with a slow time course and afterdischarge more like the effect of acetylcholine than that of L-glutamate, while the subsequent inhibitory phase appeared to be much less prominent than that found in the previous studies. With intracellular recording, the excitation caused by ibotenate was seen to be related to a depolarization of similar amplitude to that of the isoxazol to increase the rate of rise of EPSPs evoked by bipolar electrical stimulation of the cortical surface or of the n. ventralis lateralis region with concentric microelectrode. The depolarization produced by ibotenate was in several cases associated with a rise in membrane resistance as measured from the increased amplitude of hyperpolarizing pulses injected through the recording barrel. In spinal motoneurons of cats anesthetized with diallylbarbiturate, a similar, less pronounced depolarizing action of ibotenate was observed without important changes in membrane resistance. These data are in agreement with the possibility that the excitatory portion of the biphasic response of central neurons to DL-ibotenate, which is dependent on the state of anesthesia, is due at least in part to an effect similar to that exhibited by acetylcholine, i.e., through a reduction in membrane permeability to K+ relative to Na+.

Supported by the National Research Foundation and the Medical Research Council of Canada.


This study was designed to assess the effect of different doses of halothane on the averaged evoked response (AER) of freely behaving rats since halothane is one of the most commonly used general anaesthetics in this country. Stainless steel semimicroelectrodes (60 μm in diameter) were implanted under pentobarbital anesthesia in the visual and auditory cortices of rats several days prior to experimentation, along with a bipolar stimulating electrode surrounding the sural nerve. Four sets of AER's to both acoustic and visual stimuli (32 repetitions) were taken as controls and then following increasing doses of halothane at 0.25%, 0.5%, 1.0% and 2.0% administered by a vaporizer. (Fluotec 3). At each dose the sural nerve was stimulated to determine onset of anesthesia. The amplitudes of the AER's were analyzed peak to peak and consisted of an initial positive-negative spike (P1-N1) followed by a larger positive-negative-positive-negative wave (P2-N2-P3-N3). The results indicate that the auditory averaged evoked response (AER) is a more sensitive measure for detecting halothane's effect on cortical areas than the visual averaged evoked response (VARE). In general, the AER's from the auditory cortex was the most affected by the halothane averaged over all doses (59%). The later components (P2-N2-P3-N3) showed decrease related patterns of responsiveness. The next most responsive set of AER's was the AER's from the visual cortex, (32% decrease in the VARE from the auditory cortex, (38%). This last set was the only one not to exhibit increasing responsiveness in the later components. All areas showed dose-related patterns of responsiveness to the stimulus. The results from the sural nerve stimulation were that the dose level of 0.5% was the lowest dose at which the rats showed no pain-evoked response to stimulation. In all sets of recordings at this dose (0.5%), the N3 component showed an increase in amplitude over controls while other components showed mixed responses. These results indicate a relationship between anesthetic dose and cortical AER's and suggest clinical applications for monitoring level of anesthesia.


Increasing doses of d-amphetamine produce a shift in the firing pattern of neurons in the neostriatum that has been implicated in the dose-dependent transition in behavior from locomotion to focused stereotypy (Rebec and Segal, Brain Res., 1978, 150: 355). To further elucidate the neuronal mechanisms underlying the stereotypy/behavioural transition at the neuronal level, we extended our dose analysis to the nucleus accumbens. We also characterised the dose-dependent changes in firing rate produced by apomorphine. Neuronal responses immobilised, locally anesthetised rats (350-450g), was amplified and displayed by conventional means. All drugs were injected via an in-dwelling intraperitoneal catheter.

Although low doses of d-amphetamine (1.0-2.5 mg/kg) and apomorphine (0.5-1.0 mg/kg) produced a qualitatively similar depression of neuronal activity in the neostriatum and nucleus accumbens, increasing the dose caused dramatic regional differences in firing rate. Thus, whereas neurons in the neostriatum responded to 7.5 mg/kg d-amphetamine with a prolonged decrease in unit activity, the same dose produced a mirror-image inhibition in the nucleus accumbens. Increasing the dose of apomorphine (4.0 mg/kg), on the other hand, typically inhibited neuronal activity in the neostriatum but increased firing rate in the nucleus accumbens. The dose-dependent effects of d-amphetamine and apomorphine in both brain sites were reversed by either haloperidol (2.0 mg/kg) or clozapine (25-40 mg/kg).

The results indicate that the amphetamine- and apomorphine-induced behavioral transition from locomotor activity to stereotypy may be mediated, in part, by a differential dose-dependent effect on unit activity in the neostriatum and nucleus accumbens.

This research was supported by Biomedical Research Support Grant 6-F31-NS-09256-09, National Institute of Drug Abuse USPHS Grant DA-02451 from the National Institute on Drug Abuse.
ENANTIOISOMERIC INFLUENCES IN NEUROLEPTIC BINDING TO THE DOPAMINE RECEPTOR. Timothy A. Robert, Ernest A. Delagrange and Andrea N. Nagareda. Department of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, Tennessee 37601.

The stereoselectivity of the dopamine (DA) receptor has been recognized for some time. Antagonists such as cis-thiothixene, homogenates of calf caudate nucleus. Levorotatory methotrimeprazine, a single recognition parameter at the receptive site. A series of optical isomers of phenocthiaze derivatives was examined for the ability to displace 3H-DA and 3H-spiroperidol (SP) from crude homogenates of calf caudate nucleus. Levorotatory methotrimeprazine was most effective at displacing both 3H-DA and 3H-SP with IC50 values of 2 x 10^-9 and 9 x 10^-10 M, respectively. The corresponding IC50's for the dextrorotatory isomer were 3 x 10^-6 and 9 x 10^-7 M. In contrast, the dextrorotatory forms of other phenocthiazine derivatives such as promethazine were more effective at displacing 3H-DA and 3H-SP by methotrimeprazine, a large difference was observed. The receptor population labeled by 3H-SP exhibits a six-fold higher stereospecificity than that labeled by 3H-DA. These results provide direct evidence suggesting non-equivalence of 3H-DA and 3H-SP receptors in the calf caudate.

Resolved phenothiazines were the generous gifts of Wyeth Laboratories, Inc. and Rhône-Pouilene.


In order to investigate the structural requirements of dopamine receptors that mediate presynaptic events as compared to dopamine receptors that mediate postsynaptic events, the relative potencies of a series of 18 structural analogs of dopamine were determined in two models. The test series included derivatives of phenothiazine, 2-aminoethanol, benzoflquinoline and ergoline.

The agonist potencies of the test compounds at postsynaptic dopaminergic receptors were evaluated using the rotating-rat model of Ungerstedt, et al. (Brain Res. 24, 485, 1970) with the modification that a smaller volume of 6-OH-DA was used in making the unilateral substantia nigra lesions. Contralateral circulatory behavior was recorded automatically for one hour following s.c. administration of the drug. Potencies were calculated relative to apomorphine using a parallel line bioassay.

Presynaptic receptor agonists were assessed by measuring the drug-induced inhibition of 3HDA uptake in rat striatal samples following administration of gamma-butyrolactone and a DOPA decarboxylase inhibitor. The method of Walters and Roth (J. Neurochem, 26, 2 (1976)) was followed with the modification that HS0105 was used as the DOPA decarboxylase inhibitor and DOPA was determined using high performance liquid chromatography with a specific method of analysis. Potencies relative to apomorphine were calculated using a parallel line bioassay.

Seven compounds were found inactive in one or both preparations, leaving nine members of the test series with measurable potencies in both models. Although several agents appeared to have some degree of selectivity, a comparison of the rank order of the potencies of the test substances by the two models revealed a strong similarity: Spearman's correlation coefficient was 0.78 (p<0.01). These data suggest that the structural requirements of the receptors mediating rotational behavior and inhibition of DOPA accumulation are similar and therefore may involve only one receptor type.

EFFECT OF CHRONIC D-AMPHETAMINE OR β-PHENYLETHYLAMINE ON DOPAMINE BINDING IN RAT STRIATUM AND LIMBIC SYSTEM. Harold A. Robertson, Department of Pharmacology, Dalhousie University, Halifax, N. S., Canada. B3H 4H7.

D-amphetamine, a dopamine (DA)-releasing agent, produces a psychosis in man that is difficult to differentiate from paranoid schizophrenia and which is effectively controlled by neuroleptic drugs. β-Phenylethylamine (PEA), a sympathomimetic amine found in mammalian tissues, is structurally similar to amphetamine and it has been suggested that PEA may be involved in the etiology of schizophrenia. It was therefore of interest to know the effects of chronic PEA treatment on DA receptors in the rat brain.

Male Wistar rats (200 g) were injected i.p. with saline, d-amphetamine sulfate (5 mg/kg) or β-phenylethylamine-bic (50 mg/kg) daily for 24 days. Tissue pooled from 2 rats and DA receptor binding assays (using 0.2 nM 3H-spiroperidol) were carried out on striatum and the meso-limbic system. PEA treatment resulted in a consistent loss of DA receptors in the ventral tegmentum, nucleus accumbens and olfactory tubercle. Chronic treatment with either PEA or D-amphetamine produced an increase in meso-limbic 3H-spiroperidol binding in both striatum and meso-limbic system.

TREATMENT SPECIFICALLY-BOUND 3H-SPERONORIDOL

The accumulation of cyclic AMP (cAMP) in chopped brain tissue has served historically as a useful tool for identifying neurotransmitters and their interactions by examining the effects of the test substances upon the tissue content of cyclic AMP. The most striking example of this was the discovery that adenosine activates adenyl cyclase, that adenosine receptors interact with biogenic amine and adenosine receptors, and that adenosine antagonists are based upon prevention of increase in cyclic AMP in vitro by theophylline and potentiation of the inhibitory effects of the tricyclic drugs by adenosine in vivo. The tricyclic drugs produce decreases in the tissue content of ATP and increases in the content of 3'-AMP and adenosine. The increase in the tissue content of adenosine is sufficient to account for the observed increases in cyclic AMP. This work has now been extended to mice in vivo.

In order to enhance the effect of the drugs on the adenosine compounds chronic that (less than 10-fold) have been treated with mental patients. This approach results in an electroconvulsive seizure, then allowed to partially recover before freezing in isopentane. The most striking effects were seen following 3 μg/kg of M.H.G. or 40 mg/kg of H 24 h (60 days old). Five saline-injected control mice gave values for cyclic AMP and cyclic GMP of 3.68 ± 0.26 and 2.64 ± 0.38 (SEM) μg/mg of tissue. The tricyclic drugs for ATP, ADP and 5'-AMP were 8.49 ± 0.71, 1.34 ± 0.16 and 0.49 ± 0.04 μg/mg of tissue. The ATP content is more than twice the previously reported value for intact brain and indicates the effects of these techniques. Following the 1st treatment ATP increased 50% and ADP increased over 3-fold. Ninety sec. following the 2nd ECS ATP was still less half the lithium dose and increases were observed in adenosine (10-fold), cyclic AMP (3-fold) and 5'-AMP. A dose-response analysis and an exploration of other post ECS times will be discussed in relation to the probable involvement of adenosine in therapeutic and/or toxic effects of 1st. (Supported by HR 11137 and the State of Indiana).
1939
INTERACTION OF GABA MITICS WITH CENTRAL CHolinergic NEURONS.
Bernard Scatton* and Giuseppe Bartholini*(SPON: M. Le Moal). Synthé­
butyramide) on acetylcholine (ACH) levels were investigated in
various regions of the rat brain.
Muscinol (0.25 to 2 mg/kg i.p.) and SL 76 002 (10 to 400 mg/
kg i.p.) induced a dose-related increase in striatal ACh levels.
As ACh esterase activity was not affected by muscimol, SL 76 002
induced a dose-related increase in striatal ACh levels.

The enhanced activity of striatal ACh levels was also unlikely to be the consequence of GABA mimetic-indu­
ced changes in dopaminergic transmission: thus, these compounds caused a similar modification of striatal ACh levels after 6-hy­
droxydopamine induced degeneration of the nigro-striatal dopaminergic pathway. Moreover, SL 76 002 and muscimol increased striatal ACh levels to a similar extent after administration of haloperidol (2 mg/kg) or apomorphine (5 mg/kg). In addition, the unilateral lesion at substantia nigra nucleus accumbens and interpeduncular nucleus. However, the per­
centage increase was smaller in the latter areas than in striatum.
In contrast, no change of ACh levels was observed in the septo­
hippocampal system.

The present results suggest that GABA affects brain cholinergic cells in discrete brain areas.

1940
BEHAVIORAL EFFECTS OF THE NOVEL Dopamine AGONIST SKF 38393.
Paullette E. Setler, Joseph McGavitt* and Tsuneo Fujita*. Smith
SKF 38393 (2,3,4,5-tetrachydro-7,8-dihydroxy-1-phenyl-1H-3­
benzazepine) is a dopamine agonist which, in the central nervous system, stimulates selectively dopamine-linked, D2-
The complex interactions of SKF 38393 with anticholinergic drugs suggest that the behavioral effects of this agonist may more accurately reflect the consequences of activation of cyclose-coupled dopamine receptors. SKF 38393 produces contralateral rotation
when injected systemically or into the dopamine-depleted caudate
nucleus in rats with a unilateral 6-hydroxypoline-induced le­
sion of substantia nigra. In non-lesioned rats intra-caudate injection of SKF 38393 produces rotation only when the rats are
given a systemic injection of a muscarinic antagonist such as
scopolamine. SKF 38393 which, unlike most dopamine agonists,
does not produce stereotyped behavior in intact rats, does pro­
duce stereotyped behavior in rats with either unilateral or bilateral de­
pletion of dopamine and causes a mild form of stereotypy in in­
tact rats also given scopolamine. Another animal model in which
SKF 38393 produces a form of stereotyped behavior is the 6-8 day old rat, which may have a functionally weak central cholinergic system.
SKF 38393 also has little effect on the symptoms in­
duced by a combination of reserpine and -methyl-p-tyrosine.
When given with scopolamine, however, SKF 38393 reverses the postural and motor effects of acute catecholamine depletion.
SKF 38393 produces few overt effects in intact rats, but behavioral effects traditionally associated with dopamine ago­

nists may be seen in rats with denervation supersensitivity of dopamine receptors. In rats with reduced central cholinergic function. This may indicate a pharmacologic effect of SKF 38393 on cholinergic function which masks the effect of dopamine recep­
tor activation. In rats, SKF 38393 produces few overt effects but, in rats with reduced central cholinergic function, a weak activation of another type of dopamine receptor not coupled to adenylyl cyclase. This activation would be observable only when dopamine receptors are insensitive by denervation or suppression of cholinergic activity.

1941
EVIDENCE THAT AMPHETAMINE-INDUCED MYDRIASIS IN THE DOG IS DUE
PARTLY TO INHIBITION OF THE EDINGER-WESTPHAL NUCLEUS (EW) BY A
MUSCARINIC CHOLINERGIC INPUT. Lawrence G. Sharpe and Wallace B.
Pickworth. NIDA Addiction Research Center, Lexington, Kentucky
40583.
Amphetamine produces pupilary dilation in several species by the simultaneous activation of sympathetic and inhibition of parasympathetic impulses which reach the dilator and sphincter pupilae of the eye. Recent evidence indicated that microin­
jections of cholinergic muscarinic agonists in the EW caused mydriasis in the dog, suggesting that the pupilloconstrictor neurotransmitters receive a direct stimulatory input. Sharpe and Pickworth, SN Meeting, 1978).
The purpose of this study was to test the hypothesis that amphetamine produces mydriasis partly through an increased release of acetylcholine (ACH) in the EW to inhibit the pupillary light reflex path.

Five dogs were acclimated to a sling restraint. Atropine N-methyl nitrate or pilocarpine (2.73nmol in 1.0 µl) were
injected via chronically indwelling cannula in a region of the oculomotor nuclear complex that yielded pupilloconstriction to electrical stimulation. Thirty minutes after the antagonist microinjection, amphetamine (1.0 mg/kg) was infused intra­venously for 10 min. Pupillary diameter was measured photo­
graphically before and for 2 hr after the infusion.

That microinjections of atropine, but not haloperidol, pro­
duced pupilconstriction, is consistent with the hypothesis that the EW receives tonic muscarinic inhibitory input. Pre­
treatments with microinjections of atropine, but not haloper­
dol, partially and significantly antagonized amphetamine-induced mydriasis when compared to saline pretreatment (area under time action curve, P = 0.01, t = 4.35, 4 df).
Because we have previously shown that when microinjected into the EW (1) catecholamines produce miosis, (2) cholinergic mitics produce mydriasis and (3) amphetamine produces no change in pupillary diameter, we conclude that EW is not the site of action of this portion of amphetamine-induced mydriasis. One explanation is that amphetamine may increase ACh release in the EW indirectly by increasing first the release of catecholamines to excite cell bodies of those cholinergic inhibitory pathways leading to the EW.

1942
BENZODIAZEPINE BINDING IN SUBSTANTIA NIGRA: INCREASED STIMULA­
TION BY GABA AFTER STRIATONIGRAL LESIONS. Haruo Shibuya*,
Mental Health, Bethesda, MD 20025 and Dept. of Pharmacol.,
Georgetown U. School of Med. and Dent., Wash, DC 20007.
Striatonigral GABA-containing pathways were unilaterally destroyed by a discrete electrolytic lesion in the crus cerebri, rostralateral to substantia nigra (SN). In these partially destroyed, the GABA concentration of the SN from the lesioned hemi­
sphere dropped to below 40% of that on the intact side. At this time, specific [3H]GABA binding at the benzodiazepine site for its ligands (Tolman et al., Nature 274: 383, 1978). We obsenred that the [3H]diazepam binding sites remaining in the SN of the lesioned side exhibited an enhanced response to the addi­
tion of GABA to the binding assay. In membranes from the SN of the unlesioned side, 10-7M GABA did not stimulate [3H]diazepam binding, while the same GABA concentration elicited a significant increase (37%) in [3H]diazepam binding to membranes from the SN on the lesioned side was significantly reduced (by 40%) when compared with either the intact contralateral SN or with SN from unoperated control rats.

Addition of GABA (10-6 to 10-5M) to benzodiazepine binding assay preparations has been shown to increase benzodiazepine binding by increasing the apparent affinity of the benzodiazepine site for its ligands (Tolman et al., Nature 274: 383, 1978). We observed that the [3H]diazepam binding sites remaining in the SN of the lesioned side exhibited an enhanced response to the addi­
tion of GABA to the binding assay. In membranes from the SN of the unlesioned side, 10-10M GABA did not stimulate [3H]diazepam binding, while the same GABA concentration elicited a significant increase (37%) in [3H]diazepam binding to membranes from the SN on the lesioned side. At 10-6M, GABA elicited a 30% enhancement of [3H]diazepam binding in the intact SN and a 77% enhancement of [3H]diazepam binding in the SN from the lesioned hemisphere.

Acute depletion of GABA by injection of ionized (400 mg/kg.
I.p. primary hippocampal lesion, which results in a 60-70% decrease in nigral GABA) had no significant effect on the ability of GABA to enhance [3H]diazepam binding in SN. Thus it is unlikely that a reduced tissue GABA content is responsible for the diminu­
tion of the GABA effect on benzodiazepine binding. Instead, the effect which we have observed is most likely a result of the chronic loss of the GABAergic input after the unilateral substantia nigra lesion.

It has been previously shown that 2-4 weeks after electro­lytic lesions of the striatonigral pathways, there is an increase in the amount of high affinity binding sites for [3H]diazepam in SN in rats (Kubota et al., 1981). Our results suggest that these same lesions result in an enhancement of the effect of GABA on benzodiazepine binding suggests that one population of benzodiazepine receptors in SN may be closely coupled to GABA receptors in such a way that supersensitivity of GABA receptors manifests itself on the binding of benzodiazepine receptors as well.
1943 IN VITRO BINDING STUDIES WITH 3H-N-METHYL ASPARTATE. S. Robert Snodgrass (SPON: L. W. Kneisley), Dept. of Neuroscience, Children's Hospital Medical Center, and Dept. Neurology, Harvard Medical School, Boston, MA 02115.

What is the "endogenous ligand" for excitotoxins such as kainic acid (KA)? Do the large number of excitotoxins all bind to the same receptor? N-Methyl-D-aspartic acid (NMDA) is one of the most potent excitants known. McCulloch, Watkins, and others have suggested that NMDA and other excitants bind to a single receptor.

Evans et al. (Experientia 33:489, 1977) reported that microperforated concentrations of NMDA antagonized NMDA excitation in frog and rat spinal cord, but not in the NRGC. In the presence of 100 nM NMDA plus 10 mM Mg++, the effect of NMDA was greater in buffers lacking Na+ ion and 50 mM Tris acetate than in buffers containing Na+ and Tris acetate. The binding of NMDA was not altered by Ca++ but was decreased in a dose-dependent manner by Mg++. The IC50 for this Mg++ inhibition was estimated at 450 uM. Using the same membranes, buffer, and 3H-NM, no effect of Mg++ could be detected.

As with other amino acid ligands, binding reaches equilibrium very quickly. KA and glu were weak inhibitors of NMDA binding, with 3H-KA and 3H-glu showing relatively higher inhibition. The inhibition by glu was not similar to that of a D-isomer, indicating that the MAAC (M-KA-acidic) was an important antagonist. For both glu and asp, the "unnatural" D-isomers had greater potency than the L-isomers.3H-D-aspartic acid was able to block NMDA binding with a potency similar to that of 3H-D-aspartic acid and D-aspartic acid. In parallel studies, nonradioactive NMDA was a poor displacer of KA binding. The number of binding sites for NMDA appears to be somewhat less than the number of KA receptors, although both show significant regional variation and the number of binding sites for NMDA in brain varies with age.

The NRGC and surrounding areas (i.e., raphe magnus and nucleus reticularis paragigantocellularis) are responsive to nociceptive stimuli. NMDA and glutamate are important in nociception and analgesia, we microiontophoresis into the NRGC to block the increase in neuronal firing evoked by a nociceptive stimulus. MS and ME do not have a direct effect in the NRGC since neither substance blocks evoked firing. These data do not support the hypothesis that SP is a neurotransmitter in the NRGC. Therefore, we conclude that there are at least two separate nociceptive receptors which bind excitotoxins. They differ in sensitivity to NMDA and quis as ions as first noted in physiological studies.

1944 THE EFFECTS OF CHOLINE AT THE MUSCARINIC CHOLINERGIC RECEPTOR IN BRAIN. Robert C. Spath and Henry I. Yamamura, Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona 85714.

Choline is both a precursor and a metabolite of acetylcholine (ACh). Until recently, little thought was given to the possibility that choline might also interact with cholinergic receptors. We have tested choline for its ability to inhibit specific 3H-ACh binding to native cholinergic receptors in brain. Choline inhibited 3H-ACh binding with an IC50 value of 1.3 nM which is about 1000 fold less potent than ACh (IC50=1.5 uM). At 4 uM and 0.39 uM. One mChol inhibited 3H-QNB binding, shifting the QP apparent of 3H-QNB from 45 pm to 109 pm with no effect on the maximal binding capacity. The effects of choline appear to be specific to cholinergic receptors since one mChol did not significantly affect the binding of [3H]spipiroperidol or [3H]flumazenil to their receptors on brain membranes. The slopes of log-log plots for the inhibition of 3H-QNB binding by various cholinergic substances were: 0.44 for ACh, 0.73 for oxtremorine, 0.82 for choline and 1.05 for atropine. Since muscarinic agonists typically have slopes of <1, this suggests that choline may act as an agonist at muscarinic receptors in brain. Brain concentrations of choline are reported to be 15 to 30 uM, however at cholinergic synapses, the choline concentrations may be much higher. If so, it is possible that choline may interact with cholinergic receptors under normal conditions.

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1945 EFFECTS OF MORPHINE (ME), METHYLCOMINE-ENKEPHALIN (ME) AND SUBSTANCE P (SP) ON NEURAL FIRING IN THE NUCLEUS RETICULARIS GIANTOCELLULARIS (NRGC) OF THE RAT. (D. D. Spring and K. L. Haidier, Dept. of Pharmacology, Emory Univ., Atlanta, GA 30322)

The NRGC and surrounding areas (i.e., raphe magnus and nucleus reticularis paragigantocellularis) are responsive to nociceptive stimuli (Morrow and Casey, Adv. in Pain Res. and Ther. 1:595, 1976). Microinjections of low doses of morphine and enkephalin into the NRGC produce analgesia (Akaile et al., J. Neurophysiol. 47:589, 1977; Akaile et al., Neuropharmacol. 17:775, 1978). Enkephalin and SP containing neurons are found in the region of the NRGC (Hokfelt et al., Proc. Natl. Acad. Sci. USA 74:3001, 1977). Since the NRGC and surrounding areas are apparently involved in nociception and analgesia, we microiontophoresis of these substances into the NRGC to determine the effects on spontaneous neuronal firing and neuronal firing evoked by a nociceptive stimulus (i.e., foot pinch).

Male Sprague-Dawley rats (20-30 g) were anesthetized with halothane and surgically prepared with a tracheal cannula, parallysed with gallamine triethiodide, and the pO2 in the expired air was maintained between 3.5-4.7%. Local anesthetics were administered around all wound edges and on the stereotactic ear bar. In some experiments, chloral hydrate (80 mg/kg) was periodically administered through a tail vein. A fivevessel micropipette was lowered into the brain through a hole drilled in the skull. The microelectrode contained 10 nM ME, 50 nM ME, and 3 nM SP in 3% NaCl in current. In all current experiments, the micro electrode was used as a current balance barrel; the central barrel, filled with 2 M NaCl and FG, was used to record single unit activity. Most neurons tested with the nociceptive stimulus showed significant (p<0.05) delay in firing. When the nociceptive stimulus was a constant 1 mA current, the firing frequency increased and the firing frequency decreased when the nociceptive stimulus was turned off. The effects of nociceptive stimuli were blocked in the presence of 20-80 nM ME or SP. Systemically administered ME (20-80 nM ME) was ineffective in blocking the increase in firing produced by the nociceptive stimulus, but high current (up to 100 nA) microiontophoretically applied ME and ME did not. The effect of ME was systemically blocked by naloxone (1-2 mg/kg).

Systemically administered morphine apparently acts at sites other than the NRGC to block the increase in neuronal firing evoked by a nociceptive stimulus. ME and ME do not have a direct effect in the NRGC since neither substance blocked evoked firing. (Supported in part by NIDA Grant R-M-OA-0134-01.)


Destruction of the nigrostriatal bundle leads to biochemical, neurophysiological and behavioral alterations in animals. This forms the basis of a model for Parkinson's Disease in humans. Previous research utilizing this paradigm revealed that lesions lead to an increased sensitivity of striatal neurons to iontophoretically applied dopamine (DA) as well as an increased binding of [3H]-haloperidol to striatal membranes. An increased maximal stimulation of DA-sensitive adenylate cyclase in striatal homogenates from denervated striata has been observed in some studies. In the present experiments, 6-hydroxydopamine (6-OHDA; 8 mg) was injected unilaterally into the nigrostriatal bundle of male Sprague-Dawley rats weighing approximately 150 g. The animals were sacrificed at 1, 2, 3, 5, 14, and 28 days post-lesion and the corpus striata were removed for biochemical analyses. Striatal DA content was reduced by greater than 95% within 2 days following 6-OHDA administration. Nonspecific 3H-spiperoiporidal binding was defined as the binding in the presence of 2 uM (-)-butaclamol or 10 uM (+)-ADTN. No change in the binding of 3H-spiperoiporidal was observed 1 or 2 days after lesioning. However, the density of 3H-spiperoiporidal binding sites was increased by 25-50% in the denervated striata 3 or more days following 6-OHDA treatment. Basal and DA-stimulated adenylate cyclase activity were measured in fresh striatal homogenates from the same animals. A decrease in the density of DA receptors as a result of lesioning was observed. The present results support the hypothesis that the destruction of the nigrostriatal pathway leads to similar time-dependent increases in the density of DA receptors and in DA-stimulated adenylate cyclase activity. Increased 3H-spiperoiporidal binding appears to develop concomitantly with the earliest appearance of supersensitivity of DA receptors.

Supported by the National Institute of Neurological Disorders and Stroke (NS-09199).
STRESS BLOCKS NALOXONE INDUCED HYPOTHERMIA. Jane Stewart and normally participate in tonic thermoregulation. Furthermore, the naloxone in drug-naive animals arises not from a lack of tonic effects of endogenous opioids in normal animals, but rather from the somewhat stressful conditions that characterize acute testing. The frequent failure to find physiological or behavioral effects of the relatively unstressed animal has led us to conclude that the role of opiates and the endogenous opiate system in hedonic behaviors. (Supported by grants from the NIDA, Pharmaceutical Manufacturing Association Foundation and the Marquette University Committee on Research)

The specific opiate antagonist, naloxone, produced marked hypo­thermia in unstressed drug-naive rats. Rectal temperature read­ings taken 45 min after 2.5, 5, or 10 mg/kg naloxone HCl revealed a significant, dose-dependent hypothermia (> 1° C) in animals of both sexes. Drug doses were 0.1 and 0.5 ug/inj. (volume=10 nl/inj, with delivery time at 280 msec). Experiments were performed over­night for 20 hours. The highest rates of self administration (in approx. descending order) have been seen for lateral ventricle, lateral hypothalamic area, amygdala and preoptic areas. Considerably lower rates were found for the periventricular grey and septum. Finally no sig­nificant pressures were seen for cortex, cerebellum, reticular formation or fourth ventricle. Previous results indicate that 5 mg/inj Met-Enkephalin is also self-administered into LH while 4.5 mg/inj B-Endorphin resulted in an increase in total presses for each pedal.

These results point towards a communality of reward sites with­in the central nervous system and are discussed with respect to the role of opiate drugs in the endogenous opiate system.

For self-administer morphine (10 mg/kg/inj) on an FR-20 schedule of reinforcement (Kazan et al., 1967). For some of the rats, morph­ine was then replaced with methadone (2 mg/kg), LAAM (1 mg/kg), nor-LAAM (1 mg/kg) or dinor-LAAM (1 mg/kg). At least one week was allowed for the establishment of stabilized self-injection patterns. EEG recordings were stored on FM tape, and, later, EEG samples of successive REM sleep episodes which occurred between self-injections were subjected to power spectral analyses using a Nicolet MED-80 system.

During self-administration of these narcotics, the first REM sleep episode in each interinjection interval had the faster peak EEG frequency. The peak EEG frequencies of successive REM sleep episodes were best fit by a linear function. The slope of the linear EEG frequency declines reflected differences in the pharmacokinetic profiles between the narcotics. Lever pressing activity related to these changes since similar EEG frequency changes were seen in rats which received automatically delivered morphine injections. Therefore, these peak EEG frequencies may reflect a change in brain levels of the respective narcotic and/or changes in the CNS that precede "drug-seeking behavior." (Supported by NIDA Grant DA-01685.)
AGONIST INDUCED INCREASE IN BINDING AFFINITY OF \( [3 \text{H}] - \text{MUSCIMOL TO CRUDE SYNAPTIC MEMBRANES OF RAT CEREBELLM} \). Rebecca Thomas*, Affiee Gordon, and Ivan Diamond. Department of Neurology, University of California Medical School, San Francisco, Calif. 94143.

Many investigators have observed specific binding of \( \gamma \)-aminobutyric acid (GABA) agonists to receptor sites present on rat cerebellum. Binding appears to involve two sites, one of high affinity and one of lower affinity. Moreover, Toffano et al. (P.N.A. 75 (8), 4024, 1978) have demonstrated that there is a conversion from low affinity to high affinity sites by removal of an endogenous inhibitor of GABA binding. A major limitation in previous studies is that the ligand was incubated with membranes for up to 30 min. before measurement of the amount bound. In order to elucidate the mechanism of ligand binding to the GABA receptor it is necessary to study the effects of agonists and antagonists on the reaction. We have therefore been using \([3 \text{H}] - \text{muscimol} \) as a ligand. This GABA agonist has a 10-fold higher affinity for the receptor than does bicuculline and a slow off-rate which permits the use of filtration methods to measure muscimol binding. We have observed a biphasic association of \([3 \text{H}]-\text{muscimol} \) with time. Initially there is a rapid phase which is over in less than one minute. This is followed by a slower phase which appears to be linear for at least 20 min. This linear increase in binding corresponds to the initial rapid phase.

These data lend support to the evidence presented by Magati and Enna demonstrating that systemically administered Mus is independent of a specific pharmacological action at the GABA receptor.
1955
EFFECTS OF CHRONIC DESIPRAMINE TREATMENT AND α-ADRENERGIC DRUGS ON OPIATE-INDUCED DEPRESSION OF TRANSMITTER RELEASE. Loyola University Medical Center, Maywood, IL 60153.

In the present experiment, we studied the effects of acute and chronic desipramine (DMI) treatment on the electro-physiology and biochemistry of rat locus coeruleus (LC) neurons. Locus coeruleus (LC) neurons normally fire 1.73 ± 0.14 Hz (N=11) whereas after chronic DMI (5.0 mg/kg b.i.d., i.p., 7-9 days) LC impulse flow partially recovered (1.09 ± 0.24 Hz, N=12). Furthermore, after chronic DMI, LC impulse flow was continued NE uptake blockade, suggests lessened inhibition of NE neurons. Since the LC has α2-receptors on cell bodies and/or dendrites which are inhibitory to impulse flow, the present findings suggest a reduction in intracellular CaMP levels is an essential step in the induction by inhibition of opiate release.

1956
CHOLINERGIC MICROSTIMULATION OF THE PONTINE BRAIN STEM PROVOKES DESYNCRONIZED SLEEP. Emilio Vivaldi*, Robert W. Noyes, and J. Max Ruggiero. Laboratory for Sleep Research, Department of Psychiatry, Harvard Medical School, Boston MA 02115.

We have proposed that desynchronized sleep (D) results from the increase in activity of cholinergic pontine tegmental field (FTG) cells and that their recurrent collaterals play an important role in the overall population effect. The fact that local delivery of amines to the FTG produces prolonged periods of D suggests the idea of a cholinergic state generator mechanism situated in the pontine reticular formation. We have previously documented the similarity of the drug induced state to physiologically occurring D, and established that more confined and less disruptive techniques of drug delivery resulted in more consistent and specific results.

It thus seemed possible to adapt the drug delivery system to the cellular level of analysis, and thereby to determine the minimum number of cells which, when activated, could generate the whole D syndrome. We now report preliminary experience with a microchip system that combines behaviorally effective drug delivery with single unit recording capability.

Two cats were prepared for chronic EEG recording and painless head restraint. Glass micropipettes could then be stereotaxically introduced through a burr hole while sleep-wake behavior was observed and polygraphically monitored. When the tips were positioned at FTG coordinates (P 3.0, HC -7.0, L 1.5) and filled with a 1 M triethylamine hydroxide (TEA) solution, a continuous 10 Hz (N=44) in the chloral hydrate anesthetized rat. After acute DMI (1.25 or 10.0 mg/kg, i.p.), LC impulse flow decreased to 0.50 ± 0.11 Hz (N=11) whereas after chronic DMI (5.0 mg/kg b.i.d., i.p., 7-9 days) LC impulse flow partially recovered (1.09 ± 0.24 Hz, N=12). Furthermore, after chronic DMI, LC impulse flow was markedly less sensitive to the acute effects of intravenous DMI. This tolerance to DMI's effects on LC impulse flow, despite continued NE uptake blockade, suggests lessened inhibition of NE neurons. Since the LC has α2-receptors on cell bodies and/or dendrites which are inhibitory to impulse flow, the present results suggest an α2-receptor subsensitivity. Consistent with this interpretation are biochemical data showing that both 25 and 250 µg/kg doses of clonidine were equally effective in decreasing whole brain MHPG-SO4 in control rats, but only the larger dose decreased it in 12 day DMI rats. To test the sensitivity of α2-receptors (post-synaptic), prazosin (5.0 µg/kg) in a dose just sufficient to increase MHPG-SO4 in control rats was found to be similarly effective in 12 day DMI rats. These data suggest that α2-receptors become subsensitive during chronic DMI treatment but α2-receptors do not. (Research supported by USPHS Grants HH-21574, NS-10546, and MH-3831.)

1957
EFFECTS OF CHRONIC DESIPRAMINE TREATMENT AND α-ADRENERGIC DRUGS ON RAT BRAIN NORADRENERGIC NEURONS. W. Wannack, B.A. McMillan, D.C. German, and P.A. Shore. Depts. of Physiol., Psychiat., & Pharmacol., Univ. of Texas Health Science Center, Dallas, TX 75235

The purpose of the present experiment was to study the effects of acute and chronic desipramine (DMI) treatment on the electro-physiology and biochemistry of rat locus coeruleus (LC) neurons. Locus coeruleus (LC) neurons normally fire 1.73 ± 0.14 Hz (N=44) in the chloral hydrate anesthetized rat. After acute DMI (1.25 or 10.0 mg/kg, i.p.), LC impulse flow decreased to 0.50 ± 0.11 Hz (N=11) whereas after chronic DMI (5.0 mg/kg b.i.d., i.p., 7-9 days) LC impulse flow partially recovered (1.09 ± 0.24 Hz, N=12). Furthermore, after chronic DMI, LC impulse flow was markedly less sensitive to the acute effects of intravenous DMI. This tolerance to DMI's effects on LC impulse flow, despite continued NE uptake blockade, suggests lessened inhibition of NE neurons. Since the LC has α2-receptors on cell bodies and/or dendrites which are inhibitory to impulse flow, the present results suggest an α2-receptor subsensitivity. Consistent with this interpretation are biochemical data showing that both 25 and 250 µg/kg doses of clonidine were equally effective in decreasing whole brain MHPG-SO4 in control rats, but only the larger dose decreased it in 12 day DMI rats. To test the sensitivity of α2-receptors (post-synaptic), prazosin (5.0 µg/kg) in a dose just sufficient to increase MHPG-SO4 in control rats was found to be similarly effective in 12 day DMI rats. These data suggest that α2-receptors become subsensitive during chronic DMI treatment but α2-receptors do not. (Research supported by USPHS Grants HH-21574, NS-10546, and MH-3831.)

1958
OCURRENCE AND DISTRIBUTION OF RAT BRAIN 3,4-DIHYDROXYPHENYLETHYLENE GLYCOL (DHPG) DETERMINED BY GAS CHROMATOGRAPHY MASS FRAGMENTOGRAPHY. Jerry J. Warsh, Damodar D. Godse, Siu Cheung* and Peter LC. Dept. of Neurochemistry, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada, M5T 1R8.

In a number of species central nervous system (CNS) norepinephrine is preferentially metabolized to the glycol end products 3,4-dihydroxyphenylethylene glycol (DHPG) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). Laboratory based studies have been done on the occurrence and significance of MHPG in the CNS, less is known about the occurrence and biological importance of CNS DHPG. This situation stems from the lack of suitably sensitive and specific methods for quantitation of DHPG in biological samples. We describe here a sensitive and specific GC-MS assay for determination of DHPG and MHPG in biological samples and its application to the study of DHPG in rat brain.

Formic acid homogenates of whole rat brain or brain areas were subjected to enzymatic hydrolysis with acetylcholinesterase (16 h., 37°C, ph 5.9). Under basic conditions (ph 10-10.5) DHPG and MHPG were simultaneously acetylated and extracted into ethyl acetate containing acetic anhydride (0.5%). The acetylated glycol products were acylated with trifluoroacetic anhydride and quantitated by multiple ion mass fragmentography. For internal standards DHPG-2H was synthesized by reduction of 3,4-dihydroxymandelic acid with 2H-MBH. DHPG and MHPG accounted for 12% and 11%, respectively, of total DHPG and MHPG, respectively. The present data suggest that in rat brain formation and efflux of conjugated DHPG may be of magnitude as brain MHPG (87.0 ± 1.8 ng/g). Unconjugated DHPG and MHPG accounted for 87.0 ± 1.8 ng/g. Unconjugated DHPG and MHPG were simultaneously acetylated and extracted into ethyl acetate containing acetic anhydride (0.5%). The acetylated glycol products were acylated with trifluoroacetic anhydride and quantitated by multiple ion mass fragmentography. For internal standards DHPG-2H was synthesized by reduction of 3,4-dihydroxymandelic acid with 2H-MBH. DHPG and MHPG were simultaneously acetylated and extracted into ethyl acetate containing acetic anhydride (0.5%). The acetylated glycol products were acylated with trifluoroacetic anhydride and quantitated by multiple ion mass fragmentography.
1961 IDENTIFICATION OF 3H-CLONIDINE BINDING SITES IN RAT BRAIN.

F. Weinreich, M. Titeler, and P. Seeman. Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

Previous work has shown that there are two α-adrenergic receptors than can be pharmacologically differentiated (Langer, 1973; 1977; Andén et al, 1976), into a high affinity (Langer, 1973) and a low affinity norepinephrine site. The high affinity site is thought to be located pre-synaptically (Langer, 1975). A current issue is whether or not the high affinity 3H-clonidine binding site in the brain represents this same pre-synaptic α-adrenergic receptor.

The potent convulsant picrotoxinin is known to be a noncompetitive antagonist of the GABA receptor. Recently a receptor for picrotoxinin has been characterized by Olsen and his collaborators using the conformational model of picrotoxinin (Olsen et al, 1978). This model predicts that picrotoxinin and the beta isomer of 3H-dihydropicrotoxinin do not vary significantly in their binding characteristics. Consequently, we have studied the binding of these drugs in brain and central nervous system preparations.

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1965


Using the isolated frog spinal cord in conjuction with sucrose gap recording from the spinal roots it was found that piretanide (10-5 to 10-3 M) blocked the resting depolarization on the primary afferent terminals, whereas the hyperpolarizing effect of GABA on motoneurones was unchanged. The depolarizing action of α-1 receptor stimulation was also blocked while there was only variable and slight depression of glutamate responses. Piretanide also blocked the dorsal root potential (DRP) generated by ventral root stimulation. The DRP generated by dorsal root stimulation without prolonging its time course. The ventral root potential generated by dorsal root stimulation was little affected by piretanide. The action of piretanide was essentially irreversible since little recovery was seen after washing for 3 hours.

It has previously been reported (Zeuthen et al., Nature 223, 678-680, 1970) that piretanide inhibits chloride transport in intestinal epithelial cells. To examine the mechanism for the GABA blockade in our preparation intracellular recording from isolated dorsal root ganglia was initiated. Intraphoretic application of GABA produces a marked depolarization of the somata of the dorsal root ganglion cells. The depolarization is associated with a decrease in the membrane resistance and its equilibrium potential (EGABA) is 50-60 mV. Following repeated GABA applications, EGABA can be shifted towards the resting membrane potential suggesting that the ionic gradient responsible for the depolarization is being dissipated. Piretanide accelerates the shift in Eg. Since (1) is the major factor involved in the GABA response (Gray et al., J. Physiol. 225, 2-22, 1977), the effect of piretanide can be explained by an inhibition of the inward chloride pump. In addition, a reduction in the conductance change due to GABA occurred in the presence of this drug.

In summary, we have found that piretanide selectively blocks depolarizing GABA responses both by interfering with inward chloride pumping, and by reducing the chloride conductance change elicited by GABA. Such a selective drug should be valuable in separating pre- and postsynaptic inhibitory events. (Supported by GM23478 and RCDA NS00287).

1966

ALPHA-ADRENERGIC RECEPTOR SUBTYPES AND CYCLIC AMP ACCUMULATION IN MOUSE CEREBRAL CORTEX. B.B. Wolfe, J.B. Kleiner* and P.B. Molinoff. Department of Pharmacology, University of Colorado Medical Center, Denver CO 80262.

The pharmacological specificity of catecholamine-stimulated cyclic AMP accumulation in mouse cerebral cortex was determined. Both α-1 and α-2 receptors appear to increase cyclic AMP accumulation in slices of cerebral cortex. The difference between the maximal stimulation in the presence of 1-epinephrine (10-5 M) and that in the presence of (3H)-dopa (CSI) was used as a measure of α-adrenergic receptor stimulated cyclic AMP accumulation. Cortical slices from male HS mice from a colony maintained at the University of Colorado were used as a tissue source. These mice were chosen on the basis of the large α-receptor component of cyclic AMP accumulation. Typical values were 19 pmol/mg protein for α-epinephrine and 480 pmol/mg protein for α-epinephrine. The Kd values determined for a number of drugs including 1-EPI, d-EPI, clonidine, imidazoline, prazosin, yohimbine, clonidine and WB-4101.

Radioisotop binding utilizing both (3H)-WB-4101 and (3H)-clonidine was also examined. These ligands appear to bind primarily to α-1 and α-2 receptors respectively. The Kd values of a variety of agonists and antagonists were determined for the inhibition of both (3H)-WB-4101 and (3H)-clonidine binding. The values obtained for α-2 receptors were similar to those obtained with α-1 receptors. The values obtained with (3H)-WB-4101 more closely resembled the yohimbine binding. The Kd value for α-adrenergic receptor stimulated cyclic AMP accumulation in mouse cerebral cortex is not associated with the α-2 receptor as measured by (3H)-clonidine binding but may be associated with the α-1 receptor as measured by (3H)-WB-4101 binding.

To further examine this question neonatal mice were treated with α-2 adrenergic agonists and antagonists during the first 4 days of life. This was done to see if piretanide had any effect on α-receptors as measured by (3H)-WB-4101 binding. The results suggest that the α-1 receptor as measured by (3H)-WB-4101 binding is not associated with the α-2 receptor as measured by (3H)-clonidine binding. The results also indicate that the β-adrenergic receptor is a major factor involved in the GABA response (Gray et al., J. Physiol. 225, 2-22, 1977), the effect of piretanide can be explained by an inhibition of the inward chloride pump. In addition, a reduction in the conductance change due to GABA occurred in the presence of this drug.

In summary, we have found that piretanide selectively blocks depolarizing GABA responses both by interfering with inward chloride pumping, and by reducing the chloride conductance change elicited by GABA. Such a selective drug should be valuable in separating pre- and postsynaptic inhibitory events. (Supported by GM23478 and RCDA NS00287).

1964

POWER SPECTRAL ANALYSIS OF EEG AFTER ETHANOL ADMINISTRATION: CORRELATION WITH ETHANOL BLOOD LEVELS. Daniel L. Wolf* and Gerald A. Young. (Spon: L. Lemberger). The Lilly Research Laboratories, Indianapolis, IN 46206.

E-4H-pergolide binds to dopamine receptors in mammalian brain. David T. Wong,* Frank P. Bymaster,* Penny T. Lane,* Donald Kau* and Edmund C. Kornfeld.* (Spon: L. Lemberger). The Lilly Research Laboratories, Indianapolis, IN 46206.

E-4H-pergolide binds to dopamine receptors in mammalian brain. David T. Wong,* Frank P. Bymaster,* Penny T. Lane,* Donald Kau* and Edmund C. Kornfeld.* (Spon: L. Lemberger). The Lilly Research Laboratories, Indianapolis, IN 46206.

Periostin, the benzylmethyl hydroxyethylmorphine, is a potent new dopamine agonist. Pergolide causes turning in rats with unilateral nigrostriatal lesions, lowered serum prolactin, and decreased dopamine turnover in rat brain (Fuller et al., Life Sci. 24, 375, 1979). Pergolide also inhibited the binding of 3H-dopamine and 3H-spiropiperide in bovine striatal membranes with inhibitory constants (Ki) values of 7.6 and 12 nM, respectively (Moon et al., J. Pharmacol. Exp. Ther.).

Specific binding of 3H-pergolide has been demonstrated in corpus striatum and olfactory tubercle of bovine and rat brains. About 70% of the specific binding was blocked by α- and β-adrenergic and serotoninergic receptors. Both 3H-dopamine and 3H-spiperone in bovine striatal membranes with identical binding constants (Ki value) of 2.8 nM and receptor number of 370 femol/mg protein in bovine striatal Pz membranes. The corresponding values for the rat striatal Pz membranes were 3 nM and 653 femol/mg protein in dopamine agonists (amphetamine and the amphetamine derivatives, n-7 and n-17), were potent inhibitors of 3H-pergolide binding in rat striatal membranes with Ki values of 3.4, 19 and 11.3 nM, respectively. The dopamine antagonist, 3-butanol, blocked 3H-pergolide binding with a Ki value of 13 nM while its pharmacologically inactive Met-Dap binding. Butan-1-ol was a weak inhibitor of 3H-pergolide binding (Kii=225 nM). Since pergolide was over 100-fold more potent in blocking the dopamine than in blocking the serotoninergic receptors in rat slices made supersensitive to amphetamine and pergolide after nigrostriatal lesions with 6-hydroxydopamine or after chronic administration with 6-hydroxydopamine or dopamine, we conclude that pergolide produces its dopaminergic responses in vivo by acting on the postsynaptic receptors for dopamine.
Intrastriatal injections of kainic acid (K.A.) (0.2-3.8 nmol in 0.4 µl of buffered normal saline) resulted in a dose-dependent increase in glucose utilization in the ipsilateral striatum 1-2 hours after injection as determined by C-2 deoxyglucose autoradiography. Quantities of K.A. (1.9 and 3.8 nmol) sufficient to cause extensive lesions of the striatum resulted in a marked increase in glucose utilization in deeper layers of the overlying frontal neocortex 1-2 hours after injection. 1 week after injections of 1.9 or 3.8 nmol of K.A. the ipsilateral striatum showed a reduction in glucose utilization associated with a similar reduction in the number of neuronal cell bodies and choline acetyltransferase activity. Even at the lowest K.A. concentrations (0.2 nmol) there was a marked increase in glucose utilization 1-2 hours after injection in ipsilateral globus pallidus, substantia nigra, nucleus accumbens, septum, and ventral lateral geniculate. While at the higher concentrations (1.9 and 3.8 nmol) glucose utilization was markedly increased after 1-2 hours, it was not evident in ipsilateral olfactory cortex, pyriform cortex, and ventral nuclei of the thalamus as well.

 Autoradiographs following intrastriatal injection of 1 µCi of 3H-K.A. in 0.4 µl of normal saline revealed that all detectable tritium remained highly localized in the striatum for at least 2 hours after injection.

These results demonstrate that K.A. causes an increase in glucose utilization in the striatum and in some adjacent as well as far distant brain structures within 2 hours of intrastriatal injection. It appears that the increase in glucose utilization in distant sites may be mediated via direct anatomical connections with the striatum along multi-synaptic pathways rather than by diffusion or rapid axonal transport of K.A.
Rats were trained in a two-lever intracranial self-stimulation (ICSS) paradigm. Responses at the first lever delivered brain stimulation which was decreased in magnitude after every 5 responses. Responses at the second lever reset the current available to its original value. The current level at which the reset responses occurred was defined as the "reward threshold." The rate of responding at each current level during the stimulate-reset sequence was also determined. This paradigm, therefore, allowed simultaneous rate-independent and rate-dependent assessment of ICSS.

Decreased reward, as demonstrated by an elevated "reward threshold," was produced in a dose-related manner by the GABA blocking agent, picrotoxin. Similar effects could be produced by making each stimulation train less rewarding, i.e. by reducing the amount of charge delivered per stimulation. Conversely, increased reward, as indicated by a lower "reward threshold," was produced in a dose-related manner by the GABA-mimetic agent, muscimol, or by increasing the amount of charge delivered by each stimulation. Response rates were not significantly changed at any stimulation intensity following treatment with either drug.

These data suggest that the effects of picrotoxin and muscimol on ICSS are due to changed perception of reward and not to altered performance of the lever pressing task. An important role for GABA in the mediation of reward needs, therefore, to be considered.
NEUROTRANSMITTERS
1972

**N** METARAMINOL UPTAKE IN ISOLATED CEREBRAL MICROVESSELS.  
Toshiko Abe*, Kozo Abe* and Maria Spatz. Lab. Neuropath. Neuro- 
cyt. Sci., RUSCHU, NH, Bethesda, MD 20205.

Norepinephrine, which doesn't cross the blood brain barrier, 
can be taken up and metabolized in isolated cerebral microvessels 
showing features of an extraneuronal monoamine uptake. In order to elucidate further the monoamine's uptake in cerebral 
microvessels, we investigated the uptake of *N* metaraminol, a 
norepinephrine analogue which is neither metabolized by MAO nor 
Cont.

The capillary uptake of *N* metaraminol increased with the 
time incubation of the vessels, but the uptake reached a plateau 
when the concentration of *N* metaraminol was found to be saturable, because it could be inhibited by addition of unlabeled 
metaraminol in increasing concentrations to the incubation media 
confirming the labeled substance. The accumulation of *N* meta­ 
aminol in the capillaries was stimulated by K+ and Na+ and in­ 
hhibited by ouabain, KCN, DPH, adrenergic blocking agents (ima­ 
pram, propranolol), dichloroacetate and phenolthalein. Moreover, the *N* metaraminol capillary uptake was competitively 
inhibited by artemol, 5-hydroxytryptamine and cross inhibited by 
dopamine, 6-hydroxy dopamine, 5-hydroxy dopamine, 1-dopa but 
not by normetanephrine or metanephrine.

These results indicate that *N* metaraminol is taken up by K+ 
and Na+ dependent carrier mediated mechanism (which may be shared 
by other monoamines) in the cerebral microvessels. This process 
appears to be similar to the one described for neuronal mono­ 
amine uptake especially since extraneuronal uptake of amines 
was reported to be insensitive to metaraminol but sensitive to nor­ 
etanephrine and metanephrine.

1974

**INNOCYTCHOCHEMICAL LOCALIZATION OF [L-L5]-ENKEPHALIN AND SUB­ 
STANCE P IN RELATION TO NORADRENERGIC NEURONS IN AREA POSTREMA.** 
D. N. Armstrong, M. Pickel, R. J. Miller, T. H. Joh, D. J. Reis. 
Lab. of Neurobiol., Dept. of Neurology, Cornell Univ. Med. College, 
Sci., Univ. of Chicago, Chicago, IL 60637.

The Area Postrema (AP) in rat contains intrinsic cells which 
secrete dopamine beta hydroxylase (DBH) and are presumably 
noradrenergic (Torack et al., Brain Res. 61:235, 1973). We 
sought to determine by light microscopic immunocytochemistry 
whether the AP includes dopamine and adrenaline containing cells 
in addition to the noradrenergic ones. The presence and distribu­ 
tion of the peptides substance *P* (SP) and enkephalin, which 
are associated with catecholaminergic neurons in other regions 
of the brain (Pickel et al., Brain Res 160:387, 1979), were also 
examined. Specific antisera to the catecholamine synthesizing 
enzymes, tyrosine hydroxylase (TH), DBH, and phenylethanolamine 
N-methyltransferase (PNMT), and to peptides (Leu5)-enkephalin 
and substance *P* were raised in rabbit. Antisera were localized by 
the peroxidase-antiperoxidase method in 22u Vibratome sections of 
rat brain fixed by vascular perfusion with 4% paraformaldehyde. 

The enzymes CAT and GAT were assayed in the various brain 
regions. The activity of CAT was found to increase by 148% 
in the LR, 93% in the NDBB, 49% in the CN, and by 22% in the 
IF, but did not change in CM, SNR, MH, or SC. GAT activity did not 
change in the VTA, CN, SNR or IF.

The results suggest that the increase in the activity of 
the enzymes following DR stimulation takes place trans-

1975

**AMPHETAMINE EFFECTS ON SYNAPTOSOMAL DOPAMINE FORMED FROM PHENYLALANINE.** 
S. P. Bagchi, T. M. Smith* and D. P. Bagchi *Rockland Research Institute, Orangeburg, N.Y. 10962.

Amphetamine stimulates amphetamine (AA) and cocaine (CO) 
mimic the central effects of amphetamine (AMT) and all three 
effects of the non-amphetamines but not of amphetamine. To distin­ 
guish between the actions of AMT and those of AA, we have studied the 
effects of these two drugs alone and in combinations with Res, the 
synaptosomal (SR) synthesis and release of dopamine (DA) from 
phenylalanine (Phe) precursor. Phe from rat caudate nucleus was incubated with 14C-Phe in tris buffer (pH 7.4) with NaCl 
(125 mM), KCl (5 mM), MgCl2 (15 mM), pargyline (0.08 mM), glucose 
(37°C), the mixture was filtered through a 0.8 µm Milipore filter 
and the separated fractions were analyzed for labelled DA and synaptosomal level of 
14C-Phe. The results show that the addition of either Res, AA or 
CO enhanced the release of synaptosomally formed labelled DA 
to the medium. Res (1.8 micromolar) concomitantly inhibited 
the total synthesis of labelled DA, AMT and AA in 0.9 to 13 
micromolar concentration range stimulated the synthesis and their 
synthesis and release enhancing effects were comparable. In other 
experiments, AMT and AA, each in a combination with Res, were 
incubated with Phe and labelled DA synthesis/release determined. 
The results show that both AMT and AA were able to enhance fur­ 
ther the synaptosomal release heightened by Res. However, AMT 
(plus Res) was markedly effective in stimulating the total syn­ 
thesis of labelled DA over that observed in the presence of Res 
 alone but AA (plus Res) was ineffective. Furthermore, AMT had a 
greater synthesis stimulating effect in the presence than in the 
absence of Res. None of the drugs had any significant effect on 
the synaptosomal level of 14C-Phe substrate. Other results show 
that the effects of CO on the DA synthesis/release were similar 
to those of AA. The results suggest that the action of AA and CO 
can differ from that of Res on the Res responsive intrasynap­ 
tosomal DA pool at the cerebral microvesel. (Supported by NIH grants 5942485, NS06911, 
NL897A, MH 00078, and LDA-2121-01. (Substance *P* antisera 
was generously supplied by S. E. Leman.)
1976

IN VITRO EFFECTS OF pH AND PHOSPHORYLATION ON NEOSTRIATAL TYROSINE HYDROXYLASE FROM CONTROL AND HALOPERIDOL TREATED RATS. Charles Baskin* and James M. Giff (SPON: W. Stevens). Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112.

We have recently shown that the haloperidol-induced increase in the activity of tyrosine hydroxylase (TH) is pH dependent (Fed. Proc., 38, 805, 1979). This activation of TH occurs at its in vitro optimum and is the opposite of TH activity change in in vitro studies has been observed in normal conditions. It is possible that haloperidol may produce its effect by causing a phosphorylation of the enzyme. In an attempt to understand the relationship between pH, phosphorylation and the haloperidol-induced effect, neostriatal TH activity from control and haloperidol-treated (1 mg/kg) rats was assayed under normal and phosphorylating conditions at different pH values using the Nagatsu method, with 6 M NaOH as the cofactor. The apparent Vmax and Km of TH were determined at two pH values, 6.0 and 6.5. As reported previously, haloperidol treatment caused a decrease in Km in control rats but no change in Vmax for the cofactor at pH 6.5. At pH 6.0 haloperidol did not induce a change in the Km or Vmax of TH. With phosphorylating conditions at pH 6.5, TH from haloperidol-treated rats showed an increase in apparent Vmax, and a return of the Km to control values; the haloperidol-induced activation was no longer observed and the kinetics of enzyme were the same as that for normal rats. In control rats, with phosphorylating conditions at pH 6.5, an increase in Vmax of the enzyme was observed but no change in Km occurred. In contrast, neostriatal TH from control animals assayed at pH 6.0 under phosphorylating conditions showed a decrease in apparent Km but no change in Vmax. However, it should be noted that the activity of the phosphorylated enzyme at pH 6.5 is still less than the activity of TH under optimum pH conditions. These results indicate that the haloperidol-activated enzyme can be further activated by phosphorylation, which causes a return to control kinetics. Furthermore, the kinetics of phosphorylated TH from normal rats at its in vitro optimum pH are different than those at higher pH conditions. (Supported by USPHS grants GM 0579 and DA 00686.)

1977


Previous studies from this laboratory have shown that brain synaptic membranes exhibit high-affinity and high-capacity glutamate (Glu) binding sites which have many of the characteristics of the physiologic receptor for L-Glu (Michiels et al., 1974a). In addition, it has been demonstrated that the glutamic acid recognition function was associated with a glycoprotein which was solubilized from the membranes and purified to near homogeneity (Michiels et al., 1978). This high-molecular weight glycoprotein was found to be sensitive to the pH and temperature of incubation and to the pH and temperature of incubation. A microfuge centrifugation binding assay (Michiels, 1979) was modified for use in vivo in the binding of GLU to brain synaptic membranes prepared according to the method of Kanfer (1978). L-(+)-Glu binding to these membranes was determined either in vivo or buffer. For a single batch of membranes the binding activity under these conditions was almost identical. Pretreatment of the membranes with the coccineals or p-chloromercuribenzenesulfonate (PCMS) did not affect Glu binding, even though this type of treatment does block 85% of Glu transporter activity.

Glutamate binding to the synaptic membranes measured in either phosphate or Krebs and at either 25° or 37° exhibited a very rapid phase of association kinetics followed by a slower phase of binding. Half-maximal association to the rapidly-binding sites at 37° occurred in 6 - 10 sec and in 15 - 20 sec at 25°. On the basis of the kinetics of this binding interaction, the kD was estimated to be 80 - 800 nM. The dissociation rate was measured in a single binding equilibrium with apparent kD's equal to 60 - 70 nM, 200 - 2000 nM, and 3.4 nM. The most effective displacing agent found for the Glu receptor was L-glutamic acid. Glutamic acid at this concentration increased Glu binding by 50%. The nature of the synaptic membranes regardless of whether such binding was measured in phosphate or Krebs media was stable during the transport of the membrane. All of the above observations on the Glu interaction with the synaptic membranes were found to hold true also for Glu binding to the purified protein, including the lack of sensitivity to PCMS, the presence of multiple binding equilibria, and the absence of any appreciable effect of kainic acid. (Supported by GM 2297 and Institutional Biomedical Research Grant RR-50706.)

1978

THYROTROPH-RELEASING HORMONE MODULATES THE RESPONSE TO SEVERAL NEUROTYPIC TRANSMITTERS IN SOMATORENORPHIC CORTEX OF CAT. A. J. Braimam, C. R. Auker, and D. O. Carpenter. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Syntetic modulation, the nonlinear summation of responses to two transmitters, has been described for a variety of putative neurotransmitters, including the neuropeptide thyrotropin-releasing hormone (TRH). The glutamatergic neurotransmitter has been reported to specifically enhance the excitatory action of acetylcholine (Ach) on cerebral cortex neurons (Yarow, European J. Pharmacol., 14: 491-498, 1978) although Winokar and Beckman (Brain Res. 150: 205-209, 1978) were not able to confirm these results in a small sample of cells. A number of questions need to be answered concerning the role of TRH as well as neurotransmitters. Can TRH have a direct and modulatory effect on the same cell? (2) Is the modulatory effect of TRH specific for the Ach response? (3) If TRH modulates the response of one neurotransmitter, can TRH modulate the direct and modulatory effect of another neurotransmitter on that same cell? (4) Does TRH modulate specific response, e.g., the slow excitation frequently seen with Ach and histamine (Hist)? (5) Does TRH exert its effect on all cortical cells or only on specific populations of cells?

We have studied the modulatory effects of TRH on pyramidal tracts (PPT), forepaw-muscle tracts (NPT), and unidentified cells in somatotopic cortex (area 4) of cats, a source of anesthetized cats with seven-barreled microelectrodes. In each electrode, the center barrel was filled with 2M NaCl for recording single-unit activity. Each barrel was filled with 0.5M Hist, 0.5M Glutamate (Glut), or aspartate (Asp) for iontophoretic application. One barrel was filled with TRH for microiontophoretic and/or microiontophoresis injection.

The following experimental findings are pertinent to the questions posed above: (1) TRH had a direct excitatory effect on only five of 33 cells tested. In these cases, the additional finding that D- and L-glutamate are used antagonists of the glutamate and putative neurotransmitters, most particularly Asp, No presynaptic effects of Asp were seen. L-glutamate was used antagonists of the postsynaptic membrane. This antagonism occurs in the absence of observable direct effects of TRH.

1979


The effects of DL-α-aminoacidol (DLAA) on the responses to iontophoretically applied amino acids were studied using cultured dissociated fetal mouse spinal cord neurons. Intracellular recordings were obtained from spinal cord neurons grown in culture. Responses to excitatory applied iontophoretically (aspartate, ASP and glutamate, GLU) and inhibitory glycine, GLY and γ-aminobutyric acid, GABA, were diminished by tetrodotoxin blockade of spontaneous electrical activity. DLAA at DC iontophoretic currents of less than 100 nA rapidly and reversibly reduced the responses to 100 nA (0 to 100 nA) iontophoretic pulses of ASP an average of 85% (n = 24). The plot of mean antagonism of ASP by DLAA was an exponential curve with 50% reduction of the control response occurring with 20 nA of DLAA. Log-log plots of ASP dose-response curves showed no change in slope (n approx. 1) with DLAA, indicating no alteration of ASP receptor cooperativity by DLAA. In contrast to the effects of DLAA on ASP, GLU responses were reduced an average of only 22% (n = 12) by DLAA currents of 80 - 100 nA. No attenuation of GABA (n = 9) or GLY (n = 10) responses was noted with DLAA. At the iontophoretic currents used (0-100 nA) DLAA produced no change in membrane potential or membrane conductance. These results show that the DL-α-aminoacidol antagonism of ASP or GLU (n = 4), confirming reports that the DL-α-aminoacidol is the active dicarboxylic acid antagonist.

These results suggest that D- and L-glutamate are useful antagonists of dicarboxylic acid iontophoretically applied neurotransmitters, most particularly Asp. No presynaptic effects of ASP were seen, presumably DLAA antagonism occurs at the postsynaptic membrane. This antagonism occurs in the absence of observable direct effects of DLAA.
1980
IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC LOCALIZATION OF THE γ-AMINOBUTYRIC ACID SYSTEM IN FROG RETINA. C. Brandon, D.M.K. Lam*, and J.-Y. Wu. Departments of Cell Biology and Ophthalmology, Baylor College of Medicine, Houston, Texas 77030.

Several lines of evidence have implicated GABA as a neurotransmitter in the frog retina (Brain Res., 296: 476-479 (1972)) showed levels of GABA and its synthetic enzyme (glutamate decarboxylase, GAD) to be highest in the inner plexiform layer (IPL), and it was found that these levels were distributed along the presence of a high-affinity uptake system for GABA in horizontal cells and certain amacrine cells. Using a specific antiserum to the mouse brain enzyme (Brain Res., 65, 277-285 (1974)) and a Protein A-Ovalbumin-Antiperoxidase (PAP) technique, we have examined the GABA system in the frog retina by immunocytochemistry and autoradiography.

Frog (Rana pipiens) retinas were fixed by immersion in 1% formaldehyde containing 0.1% glutaraldehyde or 0.055% acrolein in 0.12M sodium phosphate buffer, pH 7.3. Tissue chopper sections (100gm thick) were treated sequentially with rabbit anti-GAD serum (1:200 dilution), protein A (50µg/ml) and PAP; all incubations were carried out in PBS containing 0.1% ovalbumin to minimize non-specific staining.

GAD-positive reaction product was observed to form five discrete laminae within the IPL. The laminae adjacent to the inner nuclear layer (INL) and the ganglion cell layer were the most dense; three evenly-spaced lighter bands were observed between them. In addition, occasional cell bodies lying within the inner third of the INL presumably amacrine cells, were filled with immunocytochemical reaction product. Slender, single processes were often seen descending from these cell bodies into the IPL.

For GABA autoradiography, retinas were incubated in vitro with 3H-GABA in frog retina, then fixed in glutaraldehyde and processed for autoradiography. Grains were observed over horizontal cells and certain amacrine cells. These results suggest that a family of amacrine cells in the frog retina uses GABA as a neurotransmitter. Ultrastructural examination of the detailed synaptic relationships of these GABA-containing neurons, thought to be involved in the formation of complex receptive field properties, will be presented.

(Supported in part by NIH grants NS-13224 and ET-02423, and a grant from the Huntington’s Chorea Foundation.)

1981
A SYNAPTOSOMAL PREPARATION FROM THE GUINEA PIG ILEUM. Clark A. Briggs*, Rose A. Schulz*, and Jack R. Cooper (Spon: James W. Pack). Dept. of Pharmacology, Yale Univ. School of Med., New Haven, Ct 06510.

The myenteric plexus of the guinea pig ileum has characteristics similar to the mammalian enteric nervous system but it is also functionally and neurochemically distinct. Further, perhaps the best known example is the similarity in potency for a wide range of narcotics in inducing analgesia in the CNS and in inhibiting acetylcholine (ACh) release from the sympathetic plexus. This similarity of the ileum to the CNS, coupled with the observations that a number of neurotransmitters and neurohormones have been shown to affect the release of ACh, prompted us to develop a synaptosomal preparation from this tissue.

After a considerable number of trials, the following procedure was adopted: Using 250-350gm Hartley guinea pigs, strips of myenteric plexus-longitudinal muscle were prepared from the entire ileum, except for the terminal 12cm, by a minor modification of the method of Paton and Vizi. High affinity choline uptake was used as a marker for synaptosomal activity during the isolation. After mincing, the strips were homogenized in 0.32M sucrose-3M sodium phosphate buffer, pH 7.2, first by Ultra­Turrax homogenization at low speed, followed by Teflon-glass homogenization. A crude fraction ("P1") containing most of the synaptosomal activity was prepared by initially centrifuging at 1,000g for 10 min, followed by centrifugation of the supernatant at 17,000g for 20 min. The synaptosomal fraction was prepared by applying the P2 to a discontinuous sucrose-Metrizamide gradient containing 3M sucrose buffer. Incubated in vitro with 14C-ACh and centrifuged at 86,000g for 30 min, an EMGOL rotor to yield a synaptosomal band with a 7 to 8 fold enrichment compared to the P2 fraction. Recovery of activity amounted to 40 to 50%. Electron microscopy of the fraction revealed a fairly homogeneous synaptosomal population with some membrane contamination but no free mitochondria.

Synaptosomal activity was indicated by a high affinity choline transport system which displayed sodium and temperature dependence and inhibition by hemicholinium-3 (HC-3) and atropine.

In preliminary experiments we have shown an inhibition by oxotremorine of [3H]-ACh release from this preparation.

1982
MUSCARINIC MODIFICATION OF VOLTAGE-SENSITIVE CURRENTS IN SYMPATHEIC NEURONS. D. A. Brown and Paul R. Adams. Dept. Physiol., and Biophys., University of Texas Medical Branch, Galveston, Texas, USA.

Bullfrog sympathetic ganglion cells in vitro were impaled with two microelectrodes (30-40 MΩ) filled with 3 M KCl and voltage-clamped. In most cases the ganglia were trypsinized and the Ringer supersaturated with 10 mM 3-5 dimethyl-2-pyrrolidone (DMP), a muscarinic agonist, 3H-muscarnine, was applied by bath-perfusion (10 nM) or by iontophoresis. The agonist produced a voltage-sensitive inward current, which was very small (<0.1 nA) at holding potentials more negative than -50mV and increased to 2-4 nA at holding potentials up to -200mV. Control steady-state current voltage (I/V) curves showed strong rectification at membrane potentials below -60mV. Currents following long (up to 0.5 sec) hyperpolarizing command-steps comprised an ohmic inward current, which was very small (time constant ~100msec) in the rectifying range of membrane potential, with an appropriate slow inverse relaxation on restoring the holding potential. The reversal potential of the slowly relaxing currents was near -80 mV. Also, the ohmic current stepping back from a hyperpolarized level was less than that seen at the onset of the hyperpolarizing step. Thus these slow inward currents probably represent deactivation and reactivation of the cell's potassium conductance. Muscarine (1) reduced the rectification of the steady-state I/V curve, (II) reduced the slow current relaxations following hyperpolarizing voltage-steps and (III) reduced the slope of the instantaneous I/V curve (negative slope of instantaneous current steps) at depolarized membrane potentials (-20 to -40 mV) by up to 70%. In contrast, the slope of both steady-state and instantaneous I/V curves at more hyperpolarized potentials (<-60 mV) was unaltered. Further, muscarine did not clearly reduce the currents generated by small (10-20 µA) depolarizing voltage steps. These observations suggest that muscarine action is to inhibit that component of the cell's potassium conductance that becomes evident at a restricted range of potentials around -35 mV, with diminishing effects at more hyperpolarized potentials. (Supported by MH grant NS-14984 and a travel grant from the Wellcome Foundation.)

1983

The molecular basis of action of alcohol (ethanol), and the neuronal components involved in its tolerance, physical dependence and withdrawal are not known. Ability of alcohol to enhance γ-aminobutyric acid (GABA) mediated transmission has been reported and withdrawal are not known. Ability of alcohol to enhance GABA actions of alcohol and also in the development of its tolerance.

The complex effects of chronic alcohol ingestion on both the actions of alcohol and also in the development of its tolerance. Perhaps the best known example is the similarity in potency for a wide range of narcotics in inducing analgesia in the CNS and in inhibiting acetylcholine (ACh) release from the sympathetic plexus. This similarity of the ileum to the CNS, coupled with the observations that a number of neurotransmitters and neurohormones have been shown to affect the release of ACh, prompted us to develop a synaptosomal preparation from this tissue.

After a considerable number of trials, the following procedure was adopted: Using 250-350gm Hartley guinea pigs, strips of myenteric plexus-longitudinal muscle were prepared from the entire ileum, except for the terminal 12cm, by a minor modification of the method of Paton and Vizi. High affinity choline uptake was used as a marker for synaptosomal activity during the isolation. After mincing, the strips were homogenized in 0.32M sucrose-3M sodium phosphate buffer, pH 7.2, first by Ultra­Turrax homogenization at low speed, followed by Teflon-glass homogenization. A crude fraction ("P1") containing most of the synaptosomal activity was prepared by initially centrifuging at 1,000g for 10 min, followed by centrifugation of the supernatant at 17,000g for 20 min. The synaptosomal fraction was prepared by applying the P2 to a discontinuous sucrose-Metrizamide gradient containing 3M sucrose buffer. Incubated in vitro with 14C-ACh and centrifuged at 86,000g for 30 min, an EMGOL rotor to yield a synaptosomal band with a 7 to 8 fold enrichment compared to the P2 fraction. Recovery of activity amounted to 40 to 50%. Electron microscopy of the fraction revealed a fairly homogeneous synaptosomal population with some membrane contamination but no free mitochondria.

Synaptosomal activity was indicated by a high affinity choline transport system which displayed sodium and temperature dependence and inhibition by hemicholinium-3 (HC-3) and atropine.

In preliminary experiments we have shown an inhibition by oxotremorine of [3H]-ACh release from this preparation.
1984
RELEASE OF ENDOGENOUS EPINEPHRINE, NOREPINEPHRINE, AND DOPAMINE FROM RAT HYPOTHALAMUS IN VITRO. Susan K. Barrese* and Richard E. Bassel* (SPGR: Ronald T. Frohman): University of Kansas, Lawrence, Kansas, 66045.

The presence and distribution of both epinephrine (EPI) and its metabolites norepinephrine (NE) and dopamine (DA) in neuroendocrine tissues and the activities and the synthesis of these neurotransmitters have suggested the existence of distinctly different adrenergic neurons in the brain. Comparative measurements of their catecholamine levels in vivo and in vitro, and tracer studies of their synthetic pathways have suggested that EPI-containing neurons may be more concentrated in the hypothalamic areas than in the brainstem areas, whereas NE-containing neurons are found more in the brainstem. In this study, we examined the activity of the amphetamine and the dopamine and the metabolism of the catecholamines in vivo.

Release of endogenous dopamine may be a more accurate indication of the extent of their activity than the conventional isotope-tracer release technique, which proposed a partial or severe deficiency in both DA (0-20%) and NE (50-60%), and involves the use of injections with antibody for the detection of multiple transmitter systems, opens the way to study a wide range of brain functions

1985

Kindling refers to the phenomenon whereby periodic electrical stimulation of the entorhinal cortex results in a behaviorally specific response initially, ultimately produces a motor seizure. Once established, this enhanced sensitivity to electrical stimulation is essential for kindling. Pharmacological studies suggest that the kindling process involves the interaction of a number of neurotransmitters. This study suggests that cholinergic mechanisms contribute to the development of kindling. We previously reported significant reductions in muscarinic cholinergic receptors in the hippocampus (Chan-Palay 1979, Chan-Palay, Palay, Wu 1979). Further experiments into the spinal cord, retina synthesis together with the uptake and transport systems for GABA, and cerebellum have been made using injections of 3H-muscimol; one of the two putative transmitter substances tested for (Chan-Palay 1979, Chan-Palay, Palay, Wu 1979). Further experiments into the spinal cord, retina synthesis together with the uptake and transport systems for GABA, and cerebellum have been made using injections of 3H-muscimol; one of the two putative transmitter substances tested for (Chan-Palay 1979, Chan-Palay, Palay, Wu 1979).

1986

In vivo injections of characterized antibodies into selected areas of the central and peripheral nervous system allow direct visualization of neurons and their synaptic relationships by specific labeling of their neurotransmitter-containing axonal varicosities. The method involves in vivo injections in the 1-25 µl range of characterized antibody for the detection of multiple transmitter systems, opens the way to study a wide range of brain functions.

1987
LOCALIZATION OF DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN THE RAT Olfactory Tubercle. A.C. Church*, B.S. Bunney and N.R. Krieger, Dept. of Pharmacology and Psychiatry, University of Pennsylvania Medical School, Philadelphia, PA, 19104, and Dept. of Psychology and Pharmacology, Yale University Medical School, New Haven, CT, 06510.

Numerous biochemical studies have demonstrated the presence of dopamine-sensitive adenylate cyclase (DSAC) within the central nervous system. The distribution of DSAC activity as a function of the neuronal layers of the olfactory tubercle has been described. The olfactory tubercle is a laminar organization, is particularly suited to neurochemical localization studies. Here we report the use of selective chemical lesions to localize DSAC within the rat olfactory tubercle. Stereotaxic injections were made directly into the tubercle with kainic acid (Kg) or with 6-OH dopamine (A8OH dopamine) (150µg, 200 µg). Using malachite green-Dawley rats (150g), unilateral injections were made with a 26 ga. needle over a 1 minute period. Animals were sacrificed 3 days later by decapitation, and the brain was rapidly removed and dissected. Homogenates of the olfactory tubercle were assayed for DSAC by the method of Keinah et al. (PNAS 62:2145, 1972). The kainic acid lesion reduced the DSAC activity by 70-90% (n=4) as compared to values from sham lesioned or unlesioned controls. Homogenates of 6-OH dopamine treated tubercles did not differ in DSAC activity from untreated controls. The lesions were assessed by histological and histofluorescence staining, the latter by either the indirect peroxidase-antiperoxidase technique and revelation with diaminobenzidine/ hydrogen peroxide. Following the immunocytochemical procedures the sections are exposed to high resolution autoradiography. The major steps for ensuring or controlling for cytochemical specificity and sensitivity in terms of antigen, antigen-antibody complexes, radioactive label through tissue preservation and visualization of neurons and their connections by subsequent autoradiography. The method involves in vivo injections in the 0.025-0.05 µl range of characterized antibody for the detection of multiple transmitter systems, opens the way to study a wide range of brain functions.

The anesthetic pentobarbital (PB) and the anticonvulsant diphenylhydantoin (DPH) have both been reported to enhance the inhibitory response to GABA, and to have depressant effects on the excitability of a number of neuronal preparations. Dorsal root ganglion cells provide a simple neuronal system which exhibits a chloride conductance increase to exogenously applied GABA. Ganglia from adult rats were placed in a continuously perfused chamber (37°C) and intracellular recordings were made with 4M K-acetate microelectrodes. All drugs were bath-applied.

PB, at concentrations 0.4-2.0x10^-4M, reversibly potentiated the amplitude (up to 160±20% of control) and duration of the GABA response in a dose-dependent manner, without having any effect on the resting potential or input resistance. At 10^-4M PB, cells were slightly depolarized and showed a small conductance increase. This is probably an expression of the GABA-aminergic property of the control, perhaps because of receptor desensitization. Additionally, PB (10^-4M) increased the accommodation to long current pulses in some cells, with little change in rheobase.

DPH, up to 2x10^-4M, did not affect the amplitude or the time course of the GABA response, nor did it change the resting potential or resistance. It did depress the amplitude and rate of rise of the action potential, as well as increase rheobase and conduction. Repetitive firing was also inhibited. Ganglion cells have variable sensitivity to tetrodotoxin (TTX) (Toshida et al. J. Neurophysiol. 41:1096-1097, 1978), and the effect of DPH was much greater on those action potentials which could be potently inhibited by TTX. Veratridine, which increases resting sodium conductance, caused a slow depolarization which was partially reversed by either TTX or DPH. Some spikes exhibited a plateau, which could be greatly prolonged by the addition of 5µM Ba^2+. DPH had no effect on this presumed calcium component (Dunlap and Fischbach. Nature. 276:837.1978) of the spike.

It is concluded that PB and DPH act through entirely different mechanisms, in this preparation. PB may significantly potentiate inhibitory GABA responses in the central nervous system, as well as decrease repetitive firing of neurons. The properties of DPH are compatible with a TTX-like inhibition of resting and voltage-sensitive sodium conductances, with no visible change in GABA inhibition. (Supported by NS 19133, Training Grant ST01-DH-02929 and a Duke Univ. Grad. School Research Award)

1990  EFFECTS OF ACETYLCHOLINE ON CULTURED MAMMALIAN CORTICAL NEURONS. Marc Dichter. Dept. Neurology, Harvard Medical School, Boston, MA 02115

Rat embryo cortical neurons grown in dissociated cell culture develop morphological, electrophysiological and pharmacological properties similar to cortical neurons in situ and the neurons form new excitatory and inhibitory synaptic connections with one another. The cultured neurons contain choline acetyltransferase (CAT) and acetylcholinesterase (AChE), both of which start at very low levels and increase dramatically during the second and third weeks in culture. Immunocytochemical staining revealed that AChE is contained in approximately 5-15% of the neurons and is not neuronal. These AChE positive neurons exhibited no specific morphology. Acetylcholine (ACH) applied by micropenetration at 1 to 500 µM produced no significant change in neuronal membrane potential, membrane conductance or action potential configuration. In approximately 50-70% of neurons, ACH produced a delayed (several seconds) and prolonged increase in spontaneous synaptic potentials, either excitatory or inhibitory, which was generated by a presynaptic mechanism. The ACH effect was blocked by concomitant application of tetrodotoxin, and therefore probably involved the activation of Na channels in axon terminals. The ACH effect was also blocked by atropine at 10-100 µM but not by nicotinic blocking agents.

It can be concluded that ACh does not act as either an excitatory or inhibitory synaptic transmitter between cortical neurons in vitro, but probably acts as a presynaptic modulator of ongoing neurotransmission.

1991  THE EFFECT OF RESERPHINE ON THE UPTAKE AND RELEASE OF TRITIATED ETA-METYTAMINE (ETA-M-TA) AND PARA-METYTAMINE (P-M-TA) IN RAT STRIATAL SLICES. L.E. Dyck* and A.A. Boulton. Psychiatric Research Division, University Hospital, Saskatoon, Sask., Canada S7N 0W8.

We have shown that slices of rat striatum possess 3H-labeled ETA-M-TA or P-M-TA can be induced to release labelled amine by 50 µM KCl and also by removal of calcium ions from the incubation medium. The latter confounds efforts to determine the calcium-dependency of the release process. Thus, to determine the source of released amine, we have examined the effect of 10 µM reserpine on the uptake of ETA-M-TA, P-M-TA, p-M-TA and m-TA-3H and on the subsequent release stimulated by 50 µM KCl, calcium removal or unlabelled ETA-M-TA, P-M-TA or DA (10 µM). Pargyline (10 µM) was present at all times and was used as a standard technique. Presumably, release stimulated from reserpine-pargyline treated slices originates from cytoplasmic sites; whereas in pargyline treated slices, release originates from synaptic sites as well. Reserpine is a well known depletor of endogenous stores of catecholamine and has been shown to reduce endogenous ETA-M-TA and P-M-TA, too. The uptakes of ETA-M-TA, P-M-TA and DA-3H, expressed as fmol/mg slice/15 min, were 324 ± 20, 373 ± 17 and 332 ± 12 (mean ± S.E.M.) in the pargyline treated slices; and were significantly reduced (P < 0.01) to 219 ± 15, 216 ± 35 and 230 ± 9, respectively, in the reserpine-pargyline treated slices. Since reserpine reduced the total uptake, the amount released in each fraction was expressed as a percentage of the total of the amounts released into each fraction and the amount left in the slices at the end of the experiment. Reserpine attenuated the release of all three whether the stimulus was 50 mM KCl or calcium removal. It could be concluded, then, that both types of stimuli caused a release of ETA-M-TA and ETA-M-TA as well as DA-3H from a vesicular site.

The effect of reserpine on release of tritiated amine by unlabelled amine (19 µM) was also investigated. The magnitudes of the release effect of unlabelled amine on preloaded ETA-M-TA and ETA-M-TA and ETA-M-TA were similar, the release of ETA-M-TA by ETA-M-TA being the least. Reserpine had no effect on the amine-induced release of ETA-M-TA, but abolished the amine-induced releases of ETA-M-TA. Reserpine did not alter the ETA-M-TA-induced release, potentiated the ETA-M-TA-induced release and attenuated the ETA-M-TA-induced release of the unlabelled amine. Support was obtained from the Medical Research Council of Canada and the Saskatchewan Health Authority.
GABA RELEASE FROM, AND ACTION IN THE PANCREAS: EFFECT ON INSULIN AND GLUCAGON RELEASE. J.C. Gerber, III and T.A. Hare, Thomas Jefferson University, Philadelphia, PA 19107.

GABA’s presence in various peripheral organs of the central nervous system is thought to be the major inhibitory neurotransmitter in the mammalian brain, as areas of the CNS contain structurally similar GABA. As in areas of the CNS, where GABA is present at a level of 1-10 nMols/mg wet weight, it is also possible that GABA may act in an inhibitory or excitatory manner in peripheral systems. Using the sensitive (lower limit of sensitivity = 1 pM) and specific (confirmed by GC/MS and radioreceptor assay) ion exchange/fluorometric assay for GABA, we have recently reported (Gerber, Hare, and Huguenard, 1979) GABA’s presence in various peripheral organs of the rat. Because GABA was present in isolated pancreatic islet tissue at a level roughly one-tenth that of brain (190 pMols/mg wet wt.) and at a similar level in catfish brockman bodies we have begun investigation of its role in endocrine pancreatic function by examining its relationship to insulin and glucagon secretion. We report here a significant (p<0.05) increase in GABA release from perfused rat islets in response to 16.7 mM glucose as compared to 2.78 mM glucose. Rabbit pancreata were found to have a CARA content very similar to that of the rat. Further, a GABA was found to facilitate insulin release into the perfusate with maximum insulin release occurring in response to 10-5 M GABA. Preliminary experiments suggest significant uptake of GABA into perfused islet tissue. (Supported in part by the HD Foundation and USPHS NIMH Grant MH28243.)
1996 BLOCKADE OF GLUTAMATE EXCITATION AND GABA INHIBITION OF BRAIN STEM NEURONS BY CURARE. R. W. Greene* and D. O. Carpenter (SPON: M. L. Varon). Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014

Curare is a competitive antagonist of the nicotinic acetylcholine receptor, where it causes a shift to the right in the dose response curve, and will block the binding of α-bungarotoxin, a specific nicotinic receptor agonist. Using curarized neurons on Axon neurons in our laboratory have demonstrated, however, that two different types of response, due to conduction increases to Na+ and Cl−, respectively, are blocked by curare when these receptors are elicited by adventitious neurotransmitters acting at different and specific receptors (J. Neurobiol. 8: 119, 1977). We suggested that these effects of curare are mediated by blockade of channels, not receptors. In these experiments we have investigated the effects of curare on the glutamate Na+ dependent excitation and GABA CI− dependent inhibition of reticulospinal neurons of the brain stem of the cat to determine if these, like the comparable responses on Ayron neurons, are blocked by curare.

The floor of the IV ventricle of cats was exposed by aspiration of the cerebellum, and recordings were made from antidromically identified reticulospinal axons to the pontine medial reticular formation. We used seven-barreled micropipettes, filled with saturated curare at pH 7, 1 M NaCl, 2 M NaCl, and 0.5 M NaAc, all at pH 4. The center barrel contained 2 M NaCl for recording. All the reticulospinal neurons encountered were silent but could be driven with a constant background current of glutamate. The glutamate concentration was determined and reversibly blocked when curare was applied by pressure injection or by ionophoresis. Sometimes this blockade was preceded by a brief period of increased excitation. The antidromic responses to glutamate were abolished by curare, which caused the transient excitation that sometimes followed curare application probably reflects blockade of natural CI− dependent inhibitory potentials. Hill et al. (J. Physiol. 187: 265, 1968) have reported that the convulsant effects of curare may result from blockade of GABA and glycine inhibition.

This research was supported by a grant to the Dept. of Neurology, Wayne State Univ. by the Detroit General Hospital Research Corp.


GABAergic axons employing glutamate decarboxylase (GAD) as a marker have identified GABAergic neuronal elements in the medial (MB) and lateral (LB) habenular nuclei. GAD activity in the LB is higher than in the MB, and was destroyed by GAD projections via the stria medullaris (SM) (Gottesfield et al., Brain Research 130: 184, 1977). The present work has been designed to more accurately localize the GABAergic system in the habenulae by immunocytochemical visualization of GAD.

Ultralight high frequency lesions were placed stereotaxically in the SM of adult rats. Following 4 months survival the animals were anaesthetized and fixed via intracardiac perfusion with solutions containing periodate-lysine-paraformaldehyde (McLean and Rakove, J. Histoch. Cytochem. 22: 1077, 1974). Coronal brain sections (50-100µm thick) were stained by the peroxidase-anti-peroxidase method of Sternberger et al. (J. Histoch. Cytochem. 22: 1077, 1974). Ascher et al. (J. Physiol. 278: 207, 1978) have applied noise analysis to identify GABAergic activity in the habenula complex. A narrow, dense streak containing GAD-positive neuron terminals was not affected by the lesioning in the MB. These nerve endings appeared in close contact with a single layer of large cell bodies which lined the third ventricle. These terminals are thus SM lesion specific.

The disappearance of GAD immunostaining from the LH in response to SM lesions corroborates the biochemical data suggesting that the SM contains GABAergic projections to the LH. Also, it provides new information on the localization of GABAergic terminals in the habenular nuclei. (Supported in part by NIMH-U54MH-265 to Z.G. and NS-13224 to J.Y.W.)

1999 PRIMARY V. SECONDARY EFFECTS OF FIGHTING ON DOPAMINE UPTAKE IN ISOLATED MICE BRAIN. M.S. Badfield, Dept. of Pathology, Medical College of Virginia, Richmond, Virginia 23298

The present study was undertaken to separate primary effects on dopamine (DA) uptake produced by fighting from secondary effects that may accompany fighting such as those resulting from motor activity and sensory stimulation. To accomplish this aim, control experiments were performed on animals not exposed to fighting (resting controls) and animals exposed to stress but not exposed to actual fighting (resting controls). To augment the power of the controls, DA uptake was studied in both the nigrostriatal (extrapyramidal) system which is known to mediate motor activity, and the mesocortical system which is altered by stress. These two DA systems were also chosen because we have just reported that fighting significantly increases turnover of DA in the nigrostriatal system but not the nigrostriatal system. (VII Int. Mtg. ISN, 1979).

Isolated male ICR mice were placed for five minutes in a combat arena (fighting animals). Of these, fighting produces increases in DA uptake in the nigrostriatal terminals, though of lesser magnitude than that noted in the fighting animals. This group alone also showed a decrease in Km and Vmax for DA uptake in the striatum. The motor/stress controls showed modest increases in Km and Vmax for DA uptake in the mesocortical terminals, though of lesser magnitude than that noted in the fighting animals. This group alone also showed a decrease in Km and Vmax for DA uptake in the striatum. The motor/stress controls showed modest increases in Km and Vmax for DA uptake in the mesocortical terminals, though of lesser magnitude than that noted in the fighting animals. This group alone also showed a decrease in Km and Vmax for DA uptake in the striatum.
**NEUROTRANSMITTERS**

**2000**

**EFFECTS OF SUBCORTICAL LESIONS ON NEOCORTICAL CHOLINERGIC MARKERS.** Stanley L. Hartgraves*, Patricia L. Menahan, Peter H. Kelly. Dept. Physiol. and Biophysics and Dept. of Anatomy, Univ. of Southern California Sch. Med., Los Angeles, CA 90033.

Experimental evidence suggests there are cholinergic pathways to the neocortex. Undertaking of the neocortex in the cat causes a large decrease in choline acetyltransferase (CAT) (Hebb et al., 1963, Nature, 198, 692). Pepau, et al. (1973, Brain Research, 57, 153) reported a decrease of acetylcholine in the neocortex of the rat following lesion of the septum. On the basis of horseradish peroxidase studies Divac (1975, Brain Research, 93, 385) has described direct projections to neocortex from neurons in the medial septum and globus pallidus region.

Here we have examined changes in the neocortex of CAT and \( [^{3}H] \) choline uptake, as markers of cholinergic neurons, one week after electrolytic lesions of the globus pallidus, medial septum and ventral thalamus. Pallidal lesions caused decreases of CAT by 58% and 42% in anterior and middle neocortical regions and a smaller decrease (by 23%) in the posterior cortex. In hippocampus CAT was unaltered. These lesions also decreased \( [^{3}H] \) choline uptake in anterior and middle neocortical regions by 35-40%. Septal lesions did not alter CAT in anterior or middle regions but caused decreases of 90% in hippocampus and 30% in posterior neocortex. Hippocampal \( [^{3}H] \) choline uptake was decreased 65% by this lesion. Lesions of the ventral thalamus caused no change in CAT activity in the regions studied (anterior and middle neocortex). These results are consistent with the hypothesis that the globus pallidus sends a diffuse network of cholinergic fibers to the neocortex, with preference to the anterior and middle regions, while the medial septal cholinergic innervation of neocortex is only to its posterior regions. Fibers from ventral thalamus contribute little or nothing to the subcortical cholinergic innervation of neocortex.

**2001**

**RECEPTOR BINDING TO ASTROGLIA CELLS: THE QNB RECEPTOR.** Fritz A. Henn, Barbara Oderfeld-Novak and Robert Kososki. U. of Ta., Iowa City, Iowa 52242.

A preliminary study of QNB binding in astroglial and synaptic somal fractions of bovine caudate revealed an enrichment of QNB binding in astroglial cells. The enzymatic profiles of the fractions suggests that both choline acetyltransferase and glutamic acid decarboxylase are enriched in the synaptic fraction along with GABA or musimol binding. The muscarinic receptor ligand QNB appears to be enriched in astroglial cells. This enrichment is not large enough to account for all QNB binding, suggesting that the ligand may bind both glial and neuronal elements. This is analogous to the diasep receptor which is clearly present in astroglia in the frontal cortex and neuronal elements in the cerebellum. Data on QNB binding has also been obtained on micro preparations of astroglia from rat hippocampus. These data support a glial localization as do lesion studies of hippocampus.

*Barbara Oderfeld-Novak was an NIH Fogarty International Fellow.*

**2002**


Age-related changes in the characteristics of accumulation of norepinephrine (NE) and choline (Ch) in chick iris in vitro were studied throughout the lifespan of the animal. We have found a specific uptake for NE at 10 days of incubation (d.i.). At this point uptake is sensitive to ouabain, demethyllysergic acid (MDA), cocaine and low sodium concentrations, but the \( V_{10} (V_{10}/V_{20}) \) value does not differ significantly from control hatch (21 d.i.). The time course of development is different for each of these individual characteristics of the uptake process, suggesting that they are selectively sensitive to changes in energy metabolism and membrane composition during ontogenesis of the chick. The kinetic values for NE uptake appear to fall into two groups; 10 d.i. to 1 month after hatch (a.h.), \( K_{m}=13-19 \mu M \), and 2 months a.h. to 2 years a.h., \( K_{m}=3.5-5 \mu M \). The \( V_{max} \) increases steadily with age up to 2 years. Other age-related changes in NE uptake are variations in sensitivity to DMI at 2 years a.h. and the appearance and increase in methanephrine sensitivity after hatch.

NE uptake is compared developmentally with Ch uptake, which is first seen at the earliest time of innervation of the iris by cholinergic fibers (4 1/2 d.i., stage 24). The \( K_{m} \) for Ch uptake does not show the same age-related changes as the \( K_{m} \) for NE uptake, but does remain stable from 1 month a.h. to 5 and 7 years of age. The \( V_{max} \) after increasing from 5 d.i. to 3 months a.h., declines at 5 and 7 years of age, paralleling changes in Ch and acetylcholine levels in the iris (see Gicobini et al., Marchi, et al., Abstracts, this meeting).

Changes in neurotransmitter uptake during development and aging reflect changes in the maturing nerve terminals. It is not yet clear if this is due to changes in molecular aspects of the uptake mechanism, or is in response to changes in the basic metabolic and structural processes of the developing and aging neuron.

(Supported by A0490-A-1039-02 and Univ. of Connecticut Research Foundation).

**2003**

**OSMOTIC LYYSIS OF BOVINE CHROMAFFIN GRANULES IN ISOTONIC SOLUTIONS OF SALTS OF WEAK ACIDS AT LOW MEDIUM pH.** Ronald W. Holz and Bruce Trock.* Dept. of Pharmacology, Univ. of Michigan Medical School., Ann Arbor, MI 48109.

Chromaffin granules have an intragranular pH of 5.5-5.7. The neutral form of weak acids such as acetic acid should be permeant and attain equal concentrations across the granule membrane. The negatively charged species should be relatively impermeant but should gain access to the granule interior by dissociation of the neutral species within the granule. The intragranular concentration of the charged species will then be determined by the local pH, the intragranular (and extragranular) concentration of neutral species, and the dissociation constant of the weak acid. One predicts that in isotonic solutions of weak acids, that granules would be stable when the medium \( pH \) is 7 because the negative species is excluded from the granule interior. When the medium \( pH \) is lowered, the intragranular concentration of both charged and uncharged species should increase. The granules should osmotically lyse and release their contents of catecholamines, ATP, and dopamine-\( \beta \)-hydroxylase (DBH) into the medium when the medium \( pH \) approaches the intragranular \( pH \). Chromaffin granules in isotonic solutions of \( K^{+} CH_{3} COO^{(-)} \), \( K^{+} CH_{3} OHCH_{2} COO^{(-)} \) (lactate), and \( K_{g} ^{-1} COOCH_{3} CH,COO^{(-)} \) (succinate) are stable at \( pH 7 \) but release their contents of catecholamines, ATP and soluble DBH when the medium \( pH \) is less than 6. The effect is inhibited by increasing the osmolality of the solution with sucrose. Granules in isotonic solutions of salts of strong acids such as \( K^{+} CH_{3} SO_{4}^{(-)} \) (methylsulfate), \( K^{+} CH_{3} OHCH_{2} SO_{3}^{-} \) (isethionate), and \( K_{g} ^{-1} COOCH_{3} CH,SO_{4}^{(-)} \) are stable between \( pH 7-0.05 \). These data provide further evidence that the intragranular \( pH \) is acidic. Supported by grants from the Michigan Heart Association and the NSF (FRNS-7824694).

*Barbara Oderfeld-Novak was an NIH Fogarty International Fellow.*

We have been studying the distribution of choline acetyl transferase (CAT) and acetylcholinesterase (AChE) in the nematode Ascaris. This and a variety of other questions have been addressed, in particular on the arrangement of different enzyme layers: hypodermis, muscle and gut plus gonads. The nervous system contains some 250 cells embedded in the hypodermis and motorneurons (Neuroscience Abstr. 4: 197, 1978). Pharmacological experiments suggest that interneurons which drive excitatory motorneurons may be localized in the hypodermis and are involved in the control of movement. Current results indicate that the distribution of enkephalin and neuropeptide Y is not correlated with the distribution of CAT and AChE activity as measured by curare (I.S. Kass, unpublished results). CAT activity in neurons, however, represents only 2% of the total activity in the animal. Most of the CAT is localized in the hypodermis and is particularly concentrated in the tip of the head. The function of ACh synthesized in hypodermis is not yet understood but it is releasable and therefore could provide additional, presumably tonic, excitatory input to muscle.

The muscle layer contains two forms of AChE separable by velocity sedimentation (SS and S5) with different KAs for ACh (SS ~ 100 μM; S5 ~ 200 μM). They are also differentially affected by detergents; The SS form is reversibly inactivated by NaCl whereas the S5 form is activated by TRZ to 100%. Comparable forms of AChE in the nematode Caenorhabditis elegans have been shown by mutant analysis to be the products of separate genes. Analysis of the distribution of separable forms with different KAs for ACh may reveal the S5 form in the front half of the animal and declines posterior to the vulva. The S5 form is more evenly distributed in the most common locomotory behavior in Ascaris, in which contraction of the vulva (2) is not blocked in zero Ca Ringer or by 20 mM Mg or 2 mM Co, precluding a presynaptic action via transmitter release from photoreceptor terminals, and is greatly reduced by 0.5 mM pentobarbitone which at similar concentrations blocks Na-mediated synaptic excitation in other preparations (3). These findings suggest that the action of KA on synapses of the DOP is specific, in that it involves a direct action of KA on post-synaptic membranes, is mediated by a Na ionophore similar to synaptic ionophores, and appears to involve binding of KA to a receptor which either has a very low affinity for GLU or which readily desensitizes or does not allow KA to act on it. Supported by NIH grants EYO 7039 to J.K. and EYO 1682 to S.T.

Propyl-2-aminobenzilate, PBCM) is a potent, specific, irreversibly binding muscarinic antagonist. In its radiolabeled form, it can be used in autoradiographic studies to localize the receptor at the microscopic level. The goal of these studies was to utilize 3H-PBCM to localize the muscarinic receptor at the electron microscopic level.

Rats were perfused with 30 ml 0.1% glutaraldehyde in buffer intracardially. The brains were rapidly removed and dissected in ice cold saline. Slices of hippocampal formation and cerebral cortex (350µ) were prepared with a tissue chopper and incubated with 5 to 25 nM concentrations of 3H-PBCM in a Kreb's Ringer solution for 15-25 min at 30°C. Some slices were preincubated with 1 µM concentrations of QNB for 3 min and incubated further with 3H-PBCM to obtain "blanks" for measurement of background values. Specific to nonspecific ratios of binding varied between about 2 to 5. The slices were rinsed with cold Ringer's solution 3 times, and further fixed with aldehydes and osmium. The slices were dehydrated with ethanol and embedded in TAAB resin.

Light microscopic autoradiography of 1µ sections revealed a limited penetration of drug into the tissue. In the present study, we confirmed the resistance of NMT neurons to the toxic action of KA in the rat and found addition that these neurons are not excited by iontophoretically-applied KA.

The "excitotoxic" hypothesis proposes that neurotransmitter amino acids exert their toxic effect through neuronal excitation. Colonnier et al. (1979) have reported that neurons of trigeminal mesencephalic nucleus (NMT) of the cat survive kainic acid (KA) injections. In the present study, we confirmed the resistance of NMT neurons to the toxic action of KA in the rat and found addition that these neurons are not excited by iontophoretically-applied KA.

In the first series of experiments, 2.5 µg of KA in 5 µl of saline were injected in the vicinity of the pontine part of NMT on one side. 5 µl vehicle was injected on the other side. Histological sections were prepared in a conventional manner. Ten and 14 days following KA injections all non-NMT neurons within a radius of 2 mm from the injection sites, including the relatively resistant cerebellar cortical granule cells, had disappeared. However NMT neurons remained and were comparable in number and appearance to those of the control saline-injected side. Thus, these observations in the rat confirm those of Colonnier et al. (1979) in the cat.

In the second series of experiments, the effect of iontophoretic application of KA was studied in NMT. This nucleus contains cell bodies of primary afferent neurons which innervate pressor reurons around the teeth and muscle spindles in the jaw closing muscles. Sixty-three NMT neurons recorded were identified by mechanical stimulation of their receptors and tested iontophotically. Thirty non-NMT neurons were tested with the same pipettes. A 0.1 M KA solution at pH 8.0 and a conventional ionophoretic technique with an automatic current "balance" were used. Recording sites were marked with fast green and verified histologically.

None of the NMT neurons responded to KA applied iontophotically, even when the currents of 100 pA or higher were used. All thirty non-NMT control neurons were activated when KA was applied with small currents (1-10 nA).

It is concluded, in the NMT, the absence of both neurotoxic and neuroexcitatory effects of KA is consistent with the "excitotoxic" hypothesis. The results also suggest that KA requires the presence of receptors to exert both its neurotoxic and neuroexcitatory actions.

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DIFFERENTIAL BINDING TO Dopamine and Neuroleptic Receptors. J. Y. Lee* and M. Goldstein. New York University Medical Center, Department of Psychiatry, 550 First Ave., New York, N.Y. 10016.

To determine whether dopamine (DA) receptors with high affinity for DA agonists have different properties than those with a high affinity for neuroleptics (neuroleptic receptors) from those with high affinity for DA agonists. The results of this study indicate that neuroleptic receptors are two distinct molecular entities: one of them is thermal stable while the other is labile.

Supported by NS 04601.

DESENSITIZING EXCITATORY RESPONSES TO Peptides, Purines, PROTONS and DRUGS REVEALED USING CULTURED MAMMALIAN NEURONS. J. F. MacDonald, J. L. Barker, D. L. Gruol, L. M. Huang*, and T. G. Smith*. Lab. of Neurophysiology, NIMHCS, NIH, Bethesda, Md. 20014

Sporal neurons derived from mouse embryos were grown in tissue culture for 4 weeks or more. Intracellular recordings using conventional and voltage clamp techniques, coupled with extracellular microiontophoreses or microperfusion were used to study the effects of taurine- methyl-phenyl-ethanol, inosine, substance P, and flurazepam on neuronal membrane properties.

Thus, evanescent excitation of central mammalian neurons is common to a wide variety of endogenous substances. A phenomenologically similar form of synaptic excitation has been reported in invertebrates. While the pharmacokinetics of flurazepam application would prevent drug-induced excitation in the intact animal, bathing medium (1-10 µM) and applied to single neurons by pressure microperfusion. Rapidly desensitizing excitatory responses on spinal neurons. Under voltage clamp the responses were associated with both an increase in inward current and membrane conductance. The responses extrapolated to an apparent reversed potential close to the peak of the spike (+20 mV), suggesting that they are due primarily to activation of sodium conductance. Responsive cells showed a non-uniform distribution of response amplitude over the cell surface.

Because iontophoresis requires charged molecules, which frequently necessitates acidification to pH 3, control pipettes containing HCl at pH 3-5 were also tested and occasionally such pipettes evoked entirely similar rapidly depolarizing, rapidly desensitizing excitatory responses. An increase in the frequency of cells responding to H+ ions was observed using pipettes containing 1M HCl or 10-100mM H+ ions. Thus, evanescent excitation of central mammalian neurons is common to a wide variety of endogenous substances. A phenomologically similar form of synaptic excitation has been reported in invertebrates. While the pharmacokinetics of flurazepam application would prevent drug-induced excitation in the intact animal, bathing medium (1-10 µM) and applied to single neurons by pressure microperfusion. Rapidly desensitizing excitatory responses on spinal neurons. Under voltage clamp the responses were associated with both an increase in inward current and membrane conductance. The responses extrapolated to an apparent reversed potential close to the peak of the spike (+20 mV), suggesting that they are due primarily to activation of sodium conductance. Responsive cells showed a non-uniform distribution of response amplitude over the cell surface.

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bicuculline has different actions on mammalian spinal cord and forebrain neurons in primary dissociated cell cultures. R.L. Macdonald, A.B. Young, L.M. Nowak. Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI 48109.

Convulsants such as bicuculline have been demonstrated to antagonize GABA-receptor inhibition in vitro and in vivo experimental preparations and it has been suggested that such antagonism forms the basis for their convulsant activity. We have investigated the action of bicuculline and GABA on forebrain and spinal cord neurons in primary dissociated cell culture and report that bicuculline produces paroxysmal depolarization in both neuronal systems with a dose-effect relationship. In spinal cord neurons and forebrain nerve cells were obtained from 13-14 or 14.5-15.5 day old fetal mice respectively and grown (as previously reported) in normal growth medium for 1-2 weeks to a sufficient density for electrophysiological study. Only neurons with spontaneous activity or with evoked action potentials were accepted for study. Paroxysmal depolarizing events (PDE) were defined as abrupt, randomly occurring depolarizations followed by collasys of action potentials and repolarization.

The percentage of forebrain neurons with PDE was a function of bicuculline concentration with about 70% at 100nM, 60% at 200nM and 100% at 500µM giving an ED50 for PDE of between 150 and 200µM. The PDE-bicuculline dose response curve was steep with PED ranging from control levels to 1000% over 8-300µM. Also as the bicuculline dose increased, time-to-peak, duration and frequency of PDE declined.

In spinal cord neurons, PDE was also produced by bicuculline but over a different dose range. PDE occurred in about 40% of neurons in 5µM, 80% in 20µM and 100% in 40µM bicuculline with a ED50 for PDE being 10µM. Also suggestive of a similar relationship between bicuculline and PDE was the control to 100% range occurring between 1-40µM. Increasing doses of bicuculline decreased PDE duration, time to peak and frequency activity in both neuronal systems. We have demonstrated that the ED50 for bicuculline-induced PDE is higher in spinal cord than in forebrain neurons in culture consistent with the findings of Young and Macdonald for ED50 for bicuculline in forebrain nerve cultures. Furthermore, the entire PDE-bicuculline dose response curve overlaps the lower 25% of the GABA-displacement curve in both preparations. This demonstrates that displacement of GABA by bicuculline in binding sites occurs at similar glycine concentrations and unique small peptides.

Thus, we have demonstrated that the ED50 for bicuculline-induced PDE is higher in spinal cord than in forebrain neurons in culture consistent with the findings of Young and Macdonald for ED50 for bicuculline in forebrain nerve cultures. Furthermore, the entire PDE-bicuculline dose response curve overlaps the lower 25% of the GABA-displacement curve in both preparations. This demonstrates that displacement of GABA by bicuculline in binding sites occurs at similar glycine concentrations and unique small peptides.


One criterion for establishing a transmitter role for a compound is to determine that a substance affecting the natural transmitter affects the putative transmitter in an identical fashion. There is substantial evidence that either glutamate or aspartate (or both) is the natural transmitter(s) of the auditory nerve. We have demonstrated the effects of GDEE and Mg on synaptically evoked responses in both neuronal systems. The data are presented in a preliminary form.

The preferential depressions of aspartate, NMDA and synaptic responses by DAA and Mg were in contrast to the preferential depression of glutamate over aspartate by GDEE while on the same unit DAA preferentially depressed the aspartate response. Again, there was a slight preference by GDEE. The data are preliminary but suggest that aspartate or an aspartate-like substance is more likely to be the transmitter of the auditory nerve than glutamate. Further experiments are being conducted to substantiate these initial observations.


Glycine is produced by an enzyme, serine trans- hydroxymethylaminotransferase, that catalyzes the conversion of serine or threonine (THR) as substrate. We suspected that THR might not be saturated with THR at normal brain tissue levels; hence we examined the effect of exogenous THR on spinal cord and brain glycine levels. Male rats were killed 1 hour after receiving 0, 50, 100, 200, or 400 mg/kg THR i.p. Cord and brain THR levels increased in a dose-dependent fashion after THR injection. The concentration of glycine in the spinal cord was significantly increased by 26% in rats receiving 400 mg/kg THR (p<0.01) and by 17% and 16% in the 200 and 100 mg/kg groups (p<0.05). Changes in brain glycine followed a similar pattern, but were not increased significantly. Doses of THR that increased cord glycine also tended to decrease its levels of the large neutral amino acid tyrosine; this indicates that THR did in fact compete with other large neutral amino acids for transport at the blood-brain barrier. THR administration (400 mg/kg) increased synaptosomal glycine levels in spinal cords by an average of 16% (from 8.53 to 9.90 nmol/mg protein), compared with a 26% increase in glycine concentrations seen in intact spinal cords.

These observations indicate that THR can be used to enhance glycine levels in rat spinal cord neurons, and to this extent, glycine synthesis may be influenced by plasma composition (that is, by the ratio of plasma THR concentration to the sum of the large neutral amino acid concentration).


Previous work has shown that the cell bodies of R3-R14, identified neurons in the abdominal ganglion of Aplysia, contain unusually high glycine concentrations and unique small peptides. These substances are candidates for release from R3-R14 as chemical messengers. We have recently located the terminals of the major axon of the Aplysia ON-unit (D-axon) to the large neuron (D1) and this has enabled us to do chemical analyses on nerves containing their preterminal processes. Using gas chromatography-mass spectrometry, we have found glycine concentrations in these nerves that are 10 times higher than in other nerves. By autoradiography, we have demonstrated a specific and rapid glycine uptake system in the axons and terminals of R3-R14.

SDS polyacrylamide gel electrophoresis demonstrated that there are small peptides in the axons and terminals of R3-R14 with molecular weights that are similar to those of the peptides found in the cell bodies. There are large quantitative differences between the gel patterns of peptides in the axons and cell bodies, consistent with peptide processing during axonal transport.

Statistical analysis of electron microscope autoradiographs demonstrated a highly significant association of axonally transported [3H]-glycine with the large osmophilic vesicles characteristic of R3-R14. Earlier work (Price et al., J. Neurobiol., in press) has shown that the radioactivity in such transport experiments is in molecular glycine, this is the first report of an association of a free amino acid with neuronal vesicles. As glycine is not osmophilic, the vesicles presumably contain peptides as well.

The presence of unique small peptides and high concentrations of free glycine in the R3-R14 terminal regions suggests that both could be released as chemical messengers at low synapses in neuronal processes. The biochemical and morphological results suggest that R3-R14 may use multiple modes of signalling to their target tissues.
SLOW EXCITATORY RESPONSE TO DOPAMINE IN APLYSIA. M. J. McCrea* and T. C. Pelchat (SPON: C. M. Woodbury). Armed Forces Radiological Research Institute, Bethesda, MD 20814.

Previous reports have demonstrated a slow excitatory response in some neurons of Aplysia to iontophoretically applied serotonin (5-HT) and GABA (Gerschenson and Paupardin-Tritsch, J. Physiol. 243: 427, 1974) characterized a slow depolarizing response to 5-HT, the A' response. This response reached peak amplitude in 10-25 sec and was almost abolished when Na+ was replaced by Tris+. Unlike the fast excitatory response to 5-HT due to an increase in sodium conductance (A response), the A' response was unchanged by 10-4M curare. Observation of a similar slow depolarizing response to GABA was made by Yarovsky and Carpenter (Sr. Res. 144: 75, 1978) in L11 of the abdominal ganglion. The underlying mechanism of this response appeared to be a conductance increase to sodium and, like the A' response to 5-HT, was curare-insensitive.

We report here that some neurons in the abdominal and buccal ganglia of Aplysia, studied under current or voltage clamp conditions, exhibit a slow depolarizing response to iontophoretically applied dopamine. The duration as well as the time-to-peak of this response is much longer than that of the fast increased sodium conductance response to dopamine which has been observed by several workers. In contrast to the fast excitatory response which peaks in 1-3 sec and lasts 10-20 sec, this slowly developing depolarization reaches 10-25 sec and lasts as long as 1-2 min. It is minimally affected by exposure to low-Cl- seawater or to 10-3M curare. The response amplitude is reduced when sucrose is substituted for NaCl. Although it has been difficult to ascertain a change in membrane conductance during the response, it behaves like a conductance increase to sodium in that the response amplitude decreases with membrane depolarization and extrapolates to zero amplitude at a potential between 0 mV and -20 mV.

These observations demonstrate another type of excitatory response to dopamine in Aplysia. The ionic mechanism of this response appears to be similar to the slow depolarizations already reported for 5-HT and GABA.


In vivo studies on the inhibitory or stimulatory action of dopamine, its agonists and antagonists on the carotid bodies of cat, dog, rabbit and rat have produced variable and conflicting results. These might occur because of the differing types of anesthesia employed and the varying levels of anesthetics achieved in the experimental animals. In the present study, decerebrate as well as chloral hydrate (35mg/kg i.p.) anesthetized rats were used to study carotid body chemoreceptor function. Injection of the dopamine agonist apomorphine (200µg/kg) by itself in the carotid artery close to the carotid body resulted in minimal excitation or no change in the carotid sinus nerve (CSN) activity or respiration. However, apomorphine potentiated the ventilatory as well as CSN response to NaCN. Chlorpromazine by itself had no significant effect on respiration and CSN activity. Dopamine (1-6µg/kg close to carotid body) produced by itself small or no excitation of respiration. Increasing the dose up to 10µg/kg did not produce any alteration in respiratory response. These results were confirmed in decerebrate rats, thus eliminating the variability, if any, due to anesthesia. Injection of high doses of dopamine (20µg/kg), however, produced by itself increase in respiration. Severance of the ninth nerve bilaterally abolished all respiratory responses to exogenous neurotropic substances. To ascertain further that the respiratory response elicited in the present study was indeed mediated by the carotid body, the ventilatory response of the cat was compared to that of the rat. As previous work has demonstrated by others recording cat CSN activity, dopamine agonists inhibited, while antagonists stimulated respiration. It thus appears that dopamine and its agonists have opposite effects on breathing responses of cat and rat, despite the similarities in morphology, innervation, neurotransmitter content, and presumed function of the carotid bodies of these different mammalian species.

2017 ACHE RELEASE FROM RAT CORTICAL SYNAPTOSOMES AND ITS RELATIONSHIP TO Na+K+-ATPase ACTIVITY. Edwin M. Meyer* and Jack R. Cooper, Dept. of Pharmacology, Yale Univ. School of Med., New Haven, CT 06510.

Since a number of problems exist with the vesicular theory of transmitter release, we are exploring the possibility that the release mechanism may involve H+ generation at the presynaptic terminal via Na+K+-ATPase activity. Accordingly, the efflux of [14C]-acetylcholine (ACh) and [14C]-choline from superfused rat cerebral-cortical synaptosomes was monitored continuously through an anhydrous scintillator flow cell. The release of [14C]-ACh but not that of [14C]-choline was increased several fold by short (10-60s) exposures to 60mM K+, 10-3M veratridine, or electrical field stimulation (20V). Each of these treatments released [14C]-ACh via a Ca2+ dependent, reversible mechanism. Ouabain (10-4-10-3M), a specific inhibitor of transport Na+K+-ATPase, also increased [14C]-ACh eflux from these cortical synaptosomes within several seconds of exposure; however, as would be predicted, ouabain-induced transmitter release was not dependent on the presence of extracellular Ca2+. Synaptosomal Na+K+-ATPase activity and [22Na]-efflux were also measured via batch or superfusion techniques, and preliminary evidence from these experiments indicated that ACh-releasing agents or treatments also inhibited synaptosomal Na+K+-ATPase activity in a manner that temporally paralleled their action on [14C]-ACh release. These preliminary results support a model which implicates changes in Na+K+-ATPase activity as a trigger for neurotransmitter release.


The nerve endings in the brain possess membrane transport mechanisms for the uptake of various substances. The compounds thought to act as transmitter characteristically are taken up by high affinity mechanism (Logan, W. J. and Snyder, S. H., Nature, 239, 297 (1971)). The accumulation of L-[35S]-cysteine into synaptosomal preparation of rat cerebral cortex was carried out with the same high affinity uptake mechanisms. (Mitra, C. H. and Smith, R. C., Society for Neuroscience. Abstract #1424, Vol 4, 448 (1978)). The high affinity uptake system appears to be associated with a unique population of nerve terminals which can be separated from the other terminals that concentrate other synaptic transmitter. Therefore, we have examined a variety of amino acids, other putative transmitters, and drugs for their ability to inhibit the high affinity uptake of L-[35S]-cysteine into crude synaptosomal preparation of rat cerebral cortex.

Twenty substances were tested as inhibitors of the uptake of L-[35S]-cysteine in crude synaptosomal preparations (P2) of rat cerebral cortex. Among cysteine analogues tested, only S-(+)-N-acetylcysteine had affinity for the uptake mechanism comparable to cysteine. L-[35S]-cysteine uptake was also potentially inhibited by norphosphine, dopamine, butyramine, L-glutamic acid, L-aspartic acid, DL-homocysteine and glutathione. L-[35S]-cysteine uptake was examined in homogenate of cerebral cortex and other regions of the rat brain. The uptake of L-[35S]-cysteine was also studied in the subcellular fractions (P1, P2 and P3) of the rat cerebral cortex homogenate. Accumulation of L-[35S]-cysteine were in the order of crude synaptosomal fraction > nuclear fractions > microsomal fractions.
2020 GABA RECEPTOR BINDING IN BOVINE RETINA: EFFECT OF FREEZING TRITON X-100, and NaClO4. Cheryl K. Mitchell* and Diana A. Backburn. (SPON: E. Simon Sears). Department of Neurobiology and Anatomy. The University of Texas Medical School at Houston, Houston, Texas 77025.

Two different types of 3H-GABA receptor binding assays were performed on retinal subcellular fractions. In one assay, fractions were pretreated by freeze-thawing, and exposure to Triton X-100 before incubations with [3H]GABA and rapid centrifugation as described by Emna and Snyder (Brain Res. 135, 174-179, 1976). These pretreatments were reported to cause a different increase in GABA receptor binding in brain synaptosomal membranes: freezing (20°C, 24 hrs)=two fold increase; treatment with 0.05% Triton X-100=five fold increase. Two retinal synaptosomal fractions were assayed: the first was enriched in large photoreceptor cell terminals (the outer plexiform layer or the OPL fraction), and the second contained conventional sized terminals (the inner plexiform layer or IPL fraction). GABA receptors from both fractions showed similar Triton sensitivity as compared to brain, with maximal stimulation noted at 0.05% Triton concentration. However, unlike brain, freeze-thawing did not result in a significant increase in apparent binding. In Triton treated material two binding sites were observed: a lower affinity site (Kp=330 nM) with the number of binding sites roughly equal in both fractions (10 pmol/gm tissue), and a higher affinity site (Kp=36 nM) limited primarily to the OPL fraction. In the second type of 3H-GABA binding assay, NaClO4 was included during the incubation period. Preliminary results indicate that, like Triton pretreatment, NaClO4 increases the specific 3H-GABA receptor binding in the OPL and IPL fractions. However, the two differences were noted: 1) the overall affinity of the GABA receptor sites is significantly lower than in Triton treated material, 2) more binding sites were observed in the IPL fractions after NaClO4 treatment than with Triton treatment. Experiments to determine the pharmacological specificity of these sites are currently underway. (This study was supported by USPH Grant E01 1655-03 and R01 HD 00088-02 to DAR.)


When administered subconvulsively (50), the excitotoxic amino acids - glutamate (Glu), aspartate (Asp) and certain analogs - selectively penetrate a specific region of the endocrine hypothalamus, the arcuate nucleus (AH), and induce disturbances in neuroendocrine function. When neurotoxic doses are employed, AH neurons are destroyed and a permanent neuroendocrine deficiency syndrome results, which includes impaired reproductive capacity and reduced size of the reproductive organs. Subtoxic doses induce reversible perturbations in endocrine functions, most of which are reversible by the injection of hormones (neuroendocrinology). Doores, T. de Gubareff*, M. Anglim*, J. Labruyere* and V. Mitchell* (1978), who recently developed a more potent excitotoxin and found each to be effective in releasing luteinizing hormone (LH), considered excitotoxic aspartate (NMA) the most promising for investigating the LH axis. When administered sc in non-toxic doses (15-40 mg/kg) to weanling or young adult male rats, NMA induces LH release by an action which is reversible, rapid, of onset, brief in duration, and is dependent upon AH neurons, since rats bearing an excitotoxin-induced AH lesion do not respond to the LH-releasing action of NMA (Price, M.T., et al., Neurosci. Abstr., 1979).

We have proposed that both the AH neurotoxic and LH-releasing actions of NMA results from interactions between excitatory receptors on the dendrobiudal surfaces of AH neurons. NMA is thought to act primarily at "Asp-prefering" receptors and it is excitatory action at such receptors is reportedly blocked specifically by the antagonist, α-aminoisobutyric acid (α-AIB). We will present evidence that αAIB blocks both the LH-releasing and the AH neurotoxic activity of NMA, whereas α-AIB, a putative neuroinhibitory transmitter, blocks the LH-releasing but not the AH neurotoxic action of NMA. This suggests that α-AIB blocks NMA specificity at its AH excitatory receptor and of NMA blocks at some other locus along this LH release pathway.

From these and other findings we are beginning to suspect that AH neurons are a major link in an LH release pathway that driven by aspartergic excitatory input to synaptic receptors on the dendrobiudal surfaces of AH neurons and is subject to GABAergic inhibition at some point between the AH neuronal perikaryon and median eminence storage terminals from which luteinizing hormone releasing hormone is secreted in response to aspartergic activation of the pathway. Supported by grants DA-02529, MH-14677, NS-09136, a Huntington's Disease Fdn. grant and R01 Award 5R01DA01884 (UM).
2026 TURNOVER OF BIOGENIC AMINES IN THE HYPOTHALAMUS OF RATS DURING UTILIZATION OF 5-HT IN THE HYPOTHALAMUS IS CORRELATED WITH PYROGEN

The binding was rapidly reversible (t1/2 at 2°C of 20 min), of a high affinity (KD of 6-13 mM) and saturable (max = about 200 pmol/mg tissue) of a pharmacological specificity was also that of a GABA receptor. Specific H-M binding was displaced by GABA (Ki about 10 mM), imidazole acetic acid (Ki about 100 mM) and (R)-bicuculline (Ki about 10 mM). The binding is non-specific (- H-bicuculline, picrotoxin and the GABA uptake inhibitor dianminobutyric acid. The specific to non-specific binding was about 6:1.

Autoregulatory studies revealed a very high density of receptors over the granule cell layer, a low level over the molecular layer and negligible receptor binding over the white matter. These results were highly reproducible and found in several experiments. A similar distribution was observed in the cerebellum (R. and A. Am. J. Neurosci. 1977). These results suggest a high localization of GABA receptors to granule cells. This suggestion is confirmed by a variety of neurochemical studies in the literature and in press. For example, removal of granule cells by viral treatment or by utilization of mutant mice results in a 60-70% loss of GABA receptor binding in the cerebellum. These results are compatible with the view that Golgi II cells as well as other cells are GABAergic.

High receptor densities were observed in many other regions of the rat brain as well. These include the substantia nigra, septum, caudate-putamen, thalamus, the substantia innominata, and parts of the dorsal horn of the spinal cord. These autoregulatory studies can contribute to localizing elements of the GABAMergic system in neural tissue. They are sensitive and provide light microscopic resolution. They could be valuable adjuncts to other histochemical methods for localizing GABA synthetase genes and GABA uptake sites. These studies were supported by USPHS grants MB25591, MH00053.

2027 INVOLVEMENT OF CYCLIC AMP IN VOLTAGE-DEPENDENT CALCIUM CURRENT ELICITED BY SEROTONIN. T. C. Pellmar and D. O. Carpenter, Department of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

In neurons of the lower left quadrant of the abdominal ganglion in Aplysia californica, a slow, inward current was observed at a holding potential of -77 mv. This current decayed extremely slow, voltage-dependent calcium current (Pellmar and Carpenter, Nature 277:483, 1976). The transmitter-induced current, studied under voltage clamp conditions, showed two regions of maximum, one at a duration of 1-4 min. The response is of maximum amplitude when the membrane potential is about +10 mV. At more depolarized and at more hyperpolarized potentials, the amplitude is smaller. The transmitter-induced calcium current is not present at potentials more hyperpolarized than -40 mV.

In the abdominal ganglion of Aplysia, perfusion of serotonin produces an increase in cyclic AMP (cAMP) (Cedar and Schwartz, J. Gen. Physiol. 60:570, 1972). It has been proposed that serotonin mediates hetero-synaptic facilitation by increasing cyclic nucleotide current through a cyclic nucleotide mechanism (Klein and Kandel, PNAS 75:3512, 1978; Shimahara and Tauc, J. Physiol. Paris 74:151, 1978). We tested the actions of cyclic nucleotides and phosphodiesterase inhibitors on this voltage-dependent calcium current. Perfusion of 10^{-5} to 10^{-4} M cAMP or dibutyryl cAMP (dBcAMP) has little effect on the amplitude and duration of the voltage-dependent response to iontophoresis of serotonin. In some experiments, a slight and transient reduction in amplitude is observed. On occasion, cAMP and dBcAMP induce a transient inward current while the membrane potential is at a depolarized potential. Adenosine (10^{-5} to 10^{-4} M) has similar effects, occasionally inducing an inward current and occasionally reducing the amplitude of the response to serotonin. The phosphodiesterase inhibitors isobutylmethylxanthine (IBMX) (1 mM), theobutylamine (2 mM), and RO 29-1724 (about 1 mM) all cause a reduction in the amplitude of the voltage-dependent calcium current elicited by serotonin. This action is reversible by washing with normal seawater.

Based on these preliminary data, it appears that cAMP does not mediate the action of serotonin to open calcium channels involved in the voltage-dependent response seen here. However, it is conceivable that the serotonin-activated conductances observed by others, including a presynaptic calcium conductance, may operate through a cAMP mechanism. The possibility that the cyclic nucleotides can modulate or directly induce a similar current cannot be excluded.
2028 SERTONIN LOCALIZATION BY FLUORESCENT TECHNIQUES IN THE BRAINS OF NORMOTENSIVE AND HYPERTENSIVE RATS.

In brains of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) were treated according to the Falck-Hillarp method suggested by de la Torre (Dynamics of Brain Monoamines I: Plenum, 1970) for histofluorescence of biogenic amines. The brains of these animals showed marked differences which depend on the degree of blood pressure change. WKY animals showed seroton in den granule deposits in all cerebellar areas of the brain not corresponding to those normally associated with serotonin deposition (i.e., the raphe system). The SHR animals showed a greater (2-3x) amount of serotonin in discrete granules spread throughout the brain. There is evidence of large amounts of serotonin in neurons and capillary endothelial cells of SHR rats. There are granule accumulations of serotonin which seem to be localized in glial cells. The majority of serotonin granules in the SHR animals, however, is within the neurons or the capillary endothelial cells. The disparity between the WKY and the SHR suggests that the brain tissues act in the hypertensive state as a storage area for serotonin, or there is an increased ability to take up serotonin. Para-chlorophenylnalene (pCPA) does not seem to abolish the serotonin fluorescence in the brain to the extent that it has been reported in peripheral tissues. The presence of serotonin in discrete large granules within cells that in addition also fluoresce with norepinephrine suggests that the SHR animals may have cells capable of containing and storing both biogenic amines at the same time. Thus a cell which may have a designated function to release only one transmitter can function as a specific receptor sites could possibly demonstrate a capability for release of "foreign" transmitters as well, whether the foreign transmitter possesses any action at postsynaptic sites remains a future question.

2029 INTERACTION OF GABAnergic AND CHOLINERGIC SYSTEMS IN RABBIT RETINA. Dianna A. Redburn and Thyon Chentanez*. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, Houston, Texas 77025.

Two synaptosome fractions were obtained from rabbit retina, one enriched in photoreceptor terminals (the outer plexiform layer, or OPL fraction), a second enriched in conventional sized terminals (the inner plexiform layer or IPL fraction). High affinity uptake sites for 3H-GABA and 3H-choline were highly enriched in the IPL fraction as compared to the OPL fraction. Uptake in both fractions was temperature and sodium dependent, and had similar affinities; however, the Vmax was four fold higher for 3H-choline and three fold higher for 3H-GABA in the IPL fraction. It is unlikely that the relatively minor GABA-ergic and cholinergic components found in the IPL were associated with contaminating synaptosomes from the IPL fraction since these systems in OPL fractions displayed unique characteristics. Specifically, in OPL fractions, the 3H-GABA uptake system was stimulated by addition of exogenous ACh. The stimulation was dose dependent and saturatable at micromolar concentrations. Increases in endogenous levels of ACh by preincubation with choline or neostigmine also stimulated 3H-GABA uptake specifically in OPL. The cholinergic stimulation was blocked by both nico- tinic and muscarinic receptor blockers, however, nicotinic blockers were 10 times more potent as measured by IC50 concen- trations. Nicotinic agonists, nicotine and DMPP mimicked the action of exogenous and exogenous ACh. If the GABAergic and cholinergic systems in the OPL are associated with photo- receptor elements then these data would be consistent with reports from other species which suggest that cholinergic cone terminals transmit indirectly to GABAergic horizontal cell terminals within the cone triad. (The work was supported by USPH Grant BYO 1655-03 and R3A 1304 00088-02 to DAR.)

2030 EFFECTS OF NEURONAL DELETIONS ON UPTAKE AND BINDING OF CEREBELLAR NEUROTTRANSMITTER CANDIDATES. Brooks N. Rohde* and W. McBride, Dept. Psych. & Biochem., Inst. of Psych. Research, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

Exposure of the cerebella to x-irradiation on a schedule from day 12 to 15 after birth (12-15x) causes a loss of late-forming granule cells while exposure to x-irradiation from day 4 to 15 (4-15x) causes the loss of granule, stellate, and basket cells. Injection of 3-aminopropyltriethoxysilane (3-APTES) causes the loss of the climbing fiber input without apparently causing damage to other cellular elements in the cerebellum. The uptake of 1.0 µM 3H-GABA (gaba) and 3H-taurine (tau) was unchanged in the crude synaptosomal fraction (P2) isolated from the cerebella of 12-15x rats relative to control values. More detailed kinetic analysis of the uptake of 3H-Glu revealed that the Km value was not changed but that the Vmax value was decreased in the 12-15x group. Decreases were seen in the P2 uptake of 1.0 µM 3H-Glu (40%), 3H-asp (30%) and 3H-GABA (40%) relative to control values. Uptake of 1.0 µM 3H-tau was not significantly different between the control and 12-15x groups. More detailed kinetic analysis of the uptake of 3H-GABA revealed that the Km value was not changed but that the Vmax value was decreased by 40% in 4-15x rats relative to control values. The uptake of 1.0 µM 3H-Glu (40%), 3H-asp, 3H-GABA and 3H-tau into the P2 fraction was not altered in the different control animals relative to control values. Sodium-independent binding of 20 nM 3H-GABA was reduced by 20% and binding of 20 nM 3H-kainic acid was reduced by 25% in synaptosomal plasma membranes (SPM) from 12-15x group relative to control values. Sodium-independent binding of 1.0 nM 3H-noradrenaline (NE) was increased by 22% in 12-15x group while binding of 1 µM [3H]dihydroxyphenylalanine (DHA) was not different from control values. The data on uptake are consistent with the idea that glu may be the excitatory neurotransmitter released from the basket cells and that the GABA may be the inhibitory transmitter released from the basket cells. However, on the basis of the uptake data, no conclusions may be drawn pertaining to tryptase by the climbing fiber terminals. The decreased sodium-independent binding of 3H-GABA and 3H-kainate may indicate a loss of GABA and glutamate receptors in the granule cell deficient cerebella. Conversely, loss of granule cells does not appear to result in a decrease of either muscarinic or beta-adrenergic receptors. (Supported by NS 13925).

2031 SOMATOSTATIN AND SUBSTANCE P INHIBIT CATECHOLAMINE SECRETION FROM GUINEA PIG CHROMAFFIN CELLS. Lorra W. Rolia*, Robert L. Perlman*, and Susan E. Leeman, Department of Physiology, Harvard Medical School, Boston, MA 02115.

We have been studying the secretion of catecholamine from purified guinea pig chromaffin cells. The chromaffin cells are isolated by collagenase digestion of guinea pig adrenal glands, followed by isopycnic centrifugation through a 50-25% (w/v) gradient of metrizamide. More than 90% of the cells in these preparations are viable chromaffin cells, as judged by fluorescence histochemistry and the exclusion of trypan blue. Purified chromaffin cells contain 400 pmol epinephrine (EPI)/mg protein (mean S.E.M., n=8). These cells accumulate [3H]-nor-epinephrine (NE) by a high-affinity uptake system (apparent Km=2 µM). During a 10 min incubation at 37°C, the cells release 15-30% of their stored EPI or their newly accumulated NE. Ace- tylycholine (ACh) causes a dose-dependent increase in the secretion of EPI and of NE; 100 µM ACh releases 105-20% of the stored catecholamines from the cells. The peptides somatostatin and substance P inhibit the secretion of catecholamine from the chromaffin cells. In twelve experiments, somatostatin (10 µM) inhibited basal catecholamine release by up to 50%, and substance P (10 µM) inhibited basal release by up to 40%. These peptides also inhibit ACh-induced catecholine secretion. Somatostatin (10-100 µM) inhibited catecholamine secretion produced by 100 µM ACh by about 55%. Half-maximal inhibition of ACh-induced catecholamine secretion was produced by 3 µM somato- statin. Substance P (10 µM) inhibited ACh-induced catecholine secretion by about 40%. Chromaffin cells were extracted with 2M acetic acid and the extracts retained immunoreactive somatostatin and immunoreactive substance P. The possible release of these peptides from the cells is being investigated.
2032 IRREVERSIBLE INHIBITION OF THE HIGH-AFFINITY CHOLINE CARRIER. B. Jane Rylett and E. Howard Colhoun*, Department of Pharmacology, University of Western Ontario, London, Ontario, Canada.

The high-affinity uptake of choline (HAC) into cholinergic nerve endings would appear to be essential to, and the rate-limiting step in, the synthesis of acetylcholine. A number of structure-activity relationships of choline have been shown to compete with choline for this carrier, with some being incorporated into the nerve ending as false transmitters. The high-affinity uptake of choline (HAC) has been found to be competitively inhibited in synaptosomes and shows an irreversible component insofar that inhibition of transport produced by this compound increases progressively with time observed at the longer incubation time thus supporting the idea of a time-dependent development of an irreversible blockade of the carrier. A corresponding decrease in the value was noted in the maximum velocity with which choline could be transported into the nerve terminals as well as an alteration in the apparent affinity with which the substrate choline binds to the carrier. A corresponding decrease in the K value was observed at the longer incubation time thus supporting the idea of a time-dependent development of an irreversible blockade of the high-affinity choline carriers. Analysis of the kinetics of inhibition produced by ChM Az with increasing substrate to show a noncompetitive type of kinetics since sufficient carrier sites could be inactivated by alkylation beyond a critical level could cause an increase in the apparent K value. Inactivation of carrier sites by alkylation to a critical level could cause an initially competitive substrate to show a noncompetitive type of kinetics since sufficient carrier sites may not always be functional to transport choline at the Vmax rate.

Supported by N.R.C. Canada.


Evidence has been presented indicating that cholinergic stimulation of the hypothalamus and septal area play an important part in the regulation of sodium and potassium in the urine (Silva-Nauto, C.R. et al. J. Physiol. 29:1971, 1976 and Saad, W.A. et al. Pharmacol. Biochem. Behav. 3:985, 1975). Recent investigations have shown the importance of the subfornical organ in the control mediation of renal sodium and potassium excretion. (Saad, W.A.et al. J. Physiol. 30:467, 1915). Further research is undertaken to provide an analysis of the degree of intensity of participation in the septal area and the subfornical organ in this control. Male Holtzman rats weighing 250-330 grams were used in these experiments. All animals had cannulas stereotaxically implanted in the septal area or in the subfornical organ. Carbachol was injected into these areas. The urine was collected over a period of 120 minutes and the concentration of Na was determined by flame photometry.

The results are shown below.

<table>
<thead>
<tr>
<th>Carbachol (ug)</th>
<th>0.01</th>
<th>0.02</th>
<th>0.1</th>
<th>0.2</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mEq/l)</td>
<td>6.4</td>
<td>0.2</td>
<td>1.4</td>
<td>2.6</td>
<td>3.5</td>
</tr>
<tr>
<td>K⁺ (mEq/l)</td>
<td>1.3</td>
<td>0.02</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In conclusion, our findings show that the subfornical organ is more sensitive than the septal area in inducing renal electrolyte excretion. Further research is under way in our laboratory with the object of studying the interaction between, hypothalaimus, septal area, and, subfornical organ in this regulation.

Supported by FAPESP.


The effect of acute and chronic glucocorticoids on the accumulation of radioactive choline (4 min at 37°C) was: caudate-putamen > anterior thalamus (anterior N.) and in the epithalamus (habenula), while smaller levels were found in the substantia nigra and in the C1 and C2 regions of the medulla oblongata. Stress produces a greater % decrease of NE levels than of E levels. These results suggest that NE neurons are involved to a greater extent than NE neurons in some responses to stress or that the synthesis rate of brain epinephrine (E) levels in the African green monkey and in the rat. In both species relatively high E levels were found in the C1 and C2 regions of the medulla oblongata and in the hypothalamus, especially ventromedial areas which include mamillary bodies, suprachiasmatic N., median eminence, lateral N.). In the monkey relatively high levels of E were also found in the thalamus (anterior N.) and in the epithalamus (habenula), while smaller levels were found in the substantia nigra and in the internal segment of the globus pallidus.

A similar regional distribution of E was observed in two human post-mortem brains. Thus, the distribution of E in human and non-human primate brain is wider than that in the rat.

To determine whether brain E participates in regulating some responses to stress we have studied the effects of immobilization stress in rats on central E and neuropepinephrine (NE) levels. Exposure of rats to stress results in a significant decrease of E and NE levels in the hypothalamus and in the C1 and C2 regions of the medulla oblongata. Stress produces a greater % decrease of E levels than of NE levels. These results suggest that E neurons are involved to a greater extent than NE neurons in some responses to stress or that the synthesis rate of brain E is slower than that of NE.

Supported by NIMH 02717 and NINDS 06801.


Using high pressure liquid chromatography we have measured epinephrine (E) levels in the brain of primates and rats. A similar regional distribution of E was observed in two human post-mortem brains. Thus, the distribution of E in human and non-human primate brain is wider than that in the rat.

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Supported by NIMH 02717 and NINDS 06801.

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The electrophysiology and pharmacology of serotonin (5-HT) responses on a cell line (TCXII) derived from the fusion of mouse neuroblastoma cells (N18TG2) with mouse sympathetic ganglion cells (Greene, L. et al Proc. Nat. Acad. Sci. USA 73: 4893, 1976) were studied. Dopamine (DA) and 5-HT elicit depolarizing, conductance increase responses in these cells (Myers, P.R., J. Cell. Physiol. 91: 103, 1977). The purpose of this study was to characterize the 5-HT response and compare the properties of the 5-HT and DA responses to determine if they are mediated through different receptors. Iontophoresis of 5-HT caused a rapid depolarization of the hybrid cell membrane potential associated with an increase in membrane conductance (Gm). The response rapidly desensitized with repeated pulses of 5-HT. These same results were obtained when DA was iontophoresed, but the cells were 10 to 100 times more sensitive to 5-HT than to DA. When equal pulses were used, the responses to 5-HT and DA appeared mediated by different receptors when examined for reversal potentials, cross-desensitization, and antagonist specificity. However, when the iontophoretic pulses were adjusted to give responses of similar amplitude, different results were obtained. DA and 5-HT cross-desensitize, while 5-HT and DA on the same cell were equal and varied from 0 to 15 nM. Perfusion of the cells with low-sodium medium reduced the 5-HT and DA responses and shifted the reversal potentials in a similar manner. Possible antagonists were bath applied in concentrations of 10 to 100 uM. No drug has been found that blocks one response and not the other. The following drugs blocked both responses: (+)-tubocurarine, chlorpromazine, hexamethonium, metegolone, bromo-1SD methothepin, cyproheptadine, clonazepam, and mersalate. Phentolamine, bromo-1SD, and cyproheptadine, applied by blunt micropipette, caused a depolarization and increase in Gm. The absence of this effect during bath application was presumably due to rapid desensitization. Since the drugs that were only bath applied may have had similar agonist effect, we have not characterized the nature of the blockade mediated by any of these drugs. Pharmacology will be reexamined by establishing dose-response curves of DA and 5-HT in the presence of varying concentrations of test drugs. In addition, the effects on membrane potential and conductance will be assessed by applying the test drugs by micropipette. The 5-HT response of TCXII appears similar to that on a number of autonomic neurons (e.g., Wallis, D. J. and North, R.A. J. Neurophysiol. 37: 1023, 1978) and on several neuroblastoma clones and neuroblastoma x glioma hybrids (MaeDermot, J. et al. Proc. Natl. Acad. Sci. USA 76: 1135, 1979). It is impossible to know how similar the 5-HT receptors are on these various preparations until more detailed pharmacology is performed on all of them.

2039 MICROIONTOPHORETIC STUDIES WITH L-DOPA AND SOME PUTATIVE NEUROTTRANSMITTERS ON THE NEURONS OF CAUDATE NUCLEUS OF RAT. J. N. Sharma and Stanley Fahn (SPON: A. S. Perman) Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

To investigate the mechanisms of action of L-dopa in Parkinson's disease the effects of microiontophoretic application of L-dopa and its metabolites and dopamine (DA) were studied on spontaneously firing and on glutamate-induced excitatory responses of caudate nucleus (CN) neurons. It was observed that while DA had only inhibitory effect and blocked the glutamate excitatory responses, L-dopa at lower dosage potentiated, whereas at higher dosage blocked the glutamate responses. The interaction between two metabolites of L-dopa, i.e. 3-O-methyltyrosine and 4-O-methyltyrosine on glutamate excitatory responses was also studied. Like L-dopa, 3-O-methyltyrosine, though at lower dosage potentiated the glutamate responses, at higher dosage failed to modify the same. The other metabolite, 4-O-methyltyrosine, did not have any effect on glutamate responses. On the other hand 3-O-methyldopamine and 4-O-methyldopamine had only blocking effect on the glutamate excitatory responses. The results suggest that L-dopa does not only act by being converted to DA at nigrostriatal nerve terminals, but may also have a direct effect on its own. The research on the modification of glutamate responses which has been suggested to be an excitatory neurotransmitter at the cortico-caudate nerve terminals. It is possible that the present observation may be related to the 'on-off' phenomenon of patients on chronic L-dopa therapy for Parkinson's disease. Furthermore, the metabolites of DA though less potent also appear to have significant and predominantly inhibitory role in the functioning of CN. It is postulated that the CN neurons have excitatory and inhibitory modulations by cortico-caudate and nigro-striatal neuronal pathways which are glutaminergic and dopaminergic in nature.

2038 IMMUNOCHEMICAL STUDIES OF ANTI-CATFISH GAD IgG. Y. Y. Thomas Su*, Jang-Yen Wu and Dominic M. K. Lam*. (Spon: D. D. Louie) Baylor College of Medicine, Houston, TX 77030

L-Glutamic acid decarboxylase from catfish brain has been purified to homogeneity by the combination of ammonium sulfate fractionation, gel filtration, calcium phosphate gel and preparative polyacrylamide gel electrophoresis. The purity of the enzyme was established by showing that several gel electrophoresis systems the enzyme migrated as a single band which contained all the enzyme activity. The antibody against purified enzyme was obtained by immunizing rabbit with injection of 80% of the enzyme. Double immunodiffusion test using this antibody against crude extract showed a sharp precipitin band. The precipitin band from this study showed enzyme activity. The inhibition of the enzyme by antibody was studied. About 65% of the enzyme was inactivated after 4 days incubation of 12 µg of enzyme with 19.2 µg of antiserum. The inhibition of the amount of the enzyme by 1.64 µg to 19.2 µg of antiserum increased from about 2% to 65% after 4 days of incubation. The enzyme activity presented in precipitate, however, increased to about 80% of the total enzyme activity remained in the reaction mixture. Microcomplement fixation of the antibody and the enzymes from different species were also studied.

Supported in part by the Retina Research Foundation (Houston), NIH grants EY 02823 and NS 13224 and a grant from Huntington's Chorea Foundation in memory of Mrs. Ruth German.
The accumulation of cyclic AMP (cAMP) in cultured BHK cells in response to β-adrenergic agonists is being studied as a model system for the study of neurotransmitter action in general. Using the adenine precipitating technique, the following points have been established.

1. In the presence of methylisobutylxanthine (MIX), the level of cyclic AMP rises rapidly in response to epinephrine (EPI) or isoproterenol (ISO), reaching a peak at the same time regardless of the concentration of agonist, during which time the cells become dispersed, but only in an important way on when the response is measured. At higher agonist concentrations the relationship between dose and response is actually reversed when the response is measured at later times, i.e., the dose-response curve is bell-shaped.

2. The equilibrium binding curve for agonists is shifted several orders of magnitude to the right of the dose-response curve, even though there is no evidence in these cells for the existence of spare receptors.

3. There is a 15 to 30 second lag between the time a supramaximal concentration of propranolol is added and the rate the effect can be observed, when the antagonist is added after the agonist.

4. These and other observations can be understood in terms of a recently developed model (Federation Proc. 38 532, 1979), the general features of which are shown below:

- ATP → cAMP → E2
- R3A → R3 + A
- R2A → R2 + A
- R1A → R1 + A

The general features of this model should be applicable to neuronal and glial cells as well as to fibroblasts, and may be applicable to responses other than the accumulation of cyclic AMP resulting from the activation of adenylyl cyclase.

**DEVELOPMENT OF GABAnergic FUNCTION IN HIPPOCAMPAL CELL CULTURES.** The source and significance of dopamine (DA) in plasma remain unknown. Furthermore, the sources of circulating dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), the major metabolites of DA, are also unknown. Extracellular DA and its metabolites may come from peripheral nerve endings, it has been suggested, that experimentally-induced alterations in brain DA metabolism may be paralleled by similar changes in plasma concentrations of DA and HVA. In order to investigate this problem further, we examined in 8 fasted (18 hrs.) male dogs the plasma DA, DOPAC and HVA responses to several drugs known to alter DA metabolism. An intravenous cannula for drug administration and blood withdrawal was inserted in a forelimb vein 90 min. prior to drug administration. After each sampling (7 x 3 ml over 3 hrs.), blood was replaced by an equal volume of saline. The dogs lay quietly throughout the experiments. DA was assayed by a radiomneutral method and DOPAC and HVA by a gas chromatographic method. Basal concentrations of plasma DA, DOPAC and HVA in dogs are 36 ± 2 pg/ml, 9.6 ± 0.6 ng/ml and 9.0 ± 0.5 ng/ml respectively. The following table summarizes the plasma DA, DOPAC and HVA responses to administration of drugs thought to alter transmission in DA neurons. The drugs examined included haloperidol, a DA receptor antagonist; pimozide, another putative DA receptor antagonist, administered in a much smaller dose but which blocked the mean activity of bromocriptine; bromocriptine, a putative DA receptor agonist.

<table>
<thead>
<tr>
<th>Drug</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol (0.5)</td>
<td>NC</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td>Pimozide (0.025)</td>
<td>NC</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td>Bromocriptine (0.015, then 0.03)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pimozide (0.025) after bromocriptine (0.015)</td>
<td>NC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bromocriptine (0.015) after pimozide (0.025)</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

NC = No change + = Increase △ = Decrease

These data are consistent with actions of pimozide and bromocriptine as mixed agonist-antagonists at DA receptors. Clearly, plasma measurements of DA, DOPAC and HVA may be used to monitor the effects of drugs on DA metabolism. However, precise sites and mechanisms of action of these drugs in altering plasma DA, DOPAC and HVA remain undefined.

(Supported by grants MA-5183 and MA-7000 from MRC of Canada)

**HISTAMINE: RECEPTORS AND NEURAL DISTRIBUTION IN MAMMALIAN CENTRAL NERVOUS SYSTEM.** Histamine H3-receptors have been successfully labeled in mammalian CNS with [3H]mepyramine (Tran et al., PNAS 75, 629, 1979). The ability to label the receptor biochemically allows us to study the potential sites of action of histamine. The receptors have wide spread distribution in mammalian peripheral organs. For example, there are abundant histamine receptor sites in the bronchial tube and the adrenal medulla. Histamine is a potent mediator of bronchial constriction and releases epinephrine from the adrenal medulla. We have found heterogeneous of histamine H3-receptors. Drug specificities differ significantly at H3-receptors in varying organs and different animal species. Regional variations of H3-receptors differ in several species and correlate poorly with endogenous histamine levels. To visualize histamine neurons we have purified histidine decarboxylase to apparent homogeneity, and inoculated rabbits and guinea pigs for antibody preparation and immunohistochemical studies.}

**DEVELOPMENT OF GABAergic FUNCTION IN HIPPOCAMPAL CELL CULTURES.** Charles R. Walker* and John H. Peacock (SPON: K. A. Kelts), Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305. Inhibitory synaptic function is mediated by γ-aminobutyric acid (GABA) in the intact hippocampus. Similarly, it is very likely that GABA mediates the abundant inhibitory synaptic activity recorded in hippocampal cell cultures. Thus we have undertaken to determine when GABAergic function occurs in mouse hippocampal cultures and to examine that function during the first 2 weeks in culture (equivalent postnatal animal age, 2-14 days).

Intracellularly recorded responses to GABA iontophoresis (0.5-1 M, pH 5) occur as early as 2 days after plating and most cells respond to GABA between days 6-14. Although responses can be elicited from distal processes, the response amplitude from processes is smaller than that recorded from the soma and proximal primary trunks. In older cultures, GABA application to the soma has been found to reversibly silence ongoing spontaneous activity.

Rapid desensitization to GABA occurs in some neurons and can mask the true magnitude of the response which ranges between 20-300 pA per nanocoulomb at a membrane potential hyperpolarized to about -100 mV. Desensitization occurred in 19/33 cells when tested with long GABA currents maintained for seconds to minutes. GABA responses are usually positive at resting potentials of -70 mV but occasionally negative responses occur. Reversal potentials to GABA can be demonstrated with both pulse and steady state iontophoresis in mouse hippocampal neuronal potentials between -30 to -55 mV. During steady GABA application, membrane conductance to test electrical pulses increases 50-60% over a -20 to -100 mV membrane potential range.

The hippocampal cultures have Na independent H3-GABA uptake that is neuronally specific as shown by autoradiography and by the use of the inhibitors GABA and a-aminobutyric acid. The presence of Na independent uptake has been found in culture with intact cells using both "H-GABA and "H-muscimol. The K, for muscimol binding is approximately 2 mM but the kinetics for "H-GABA have been difficult to determine because of rapid dissociation of GABA from the receptor. In GABA receptors are present and functional) at early times in culture. The diversity of GABA responses in terms of geographical distribution, polarity, and desensitization may reflect differences in cell type or differentiated functions in these young cultures and pose questions for further study. (Supported by NIH grants NS 12151 and NS 07012)
**2046**

**EFFECTS OF LITHIUM ON EXCITATORY RESPONSES TO SEROTONIN AND DOPAMINE IN APLYSIA.** A. M. Williamson*, T. C. Pellmar, and D. O. Carpenter. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

In Aplysia, serotonin and dopamine are neuronal transmitters that play important roles in various behaviors. Serotonin is involved in feeding and attending to conspecifics, whereas dopamine is associated with learning and emotions. Lithium, a well-known mood stabilizer, has been shown to affect the activity of these neurotransmitters.

This study investigated the effects of lithium on the excitatory responses to serotonin and dopamine in Aplysia. The researchers observed that lithium reverses the reduction in amplitude of the responses to both neurotransmitters and that these responses are modulated by a sodium conductance increase to serotonin. A' responses of Gerschenfeld and Paupardin-Tritsch were also reduced in amplitude at both lithium concentrations. Those reversible. The fast conductance increases due to serotonin and dopamine are identical, then these ionophores should respond similarly to sodium substitutes in artificial seawater with lithium (Li-seawater) on the excitatory responses of morphologically identified mammalian, neocortical neurons. This suggests that there are common ionophores for each class of ionic response (Swann and Carpenter, Nature 236: 751, 1973). If the ionophores mediating the increases to several neurotransmitters are attributable to the pharmacologic action of these agents.

The observation that lithium affects the fast excitatory responses to dopamine and serotonin in a similar manner supports the hypothesis that they are mediated by the same ionophore. Since lithium acts differently on the slow excitatory response to serotonin, a different ionophore may be involved.

**2047**

**RESPONSES OF MORPHOLOGICALLY IDENTIFIED MAMMALIAN, NEOCORTICAL NEURONS TO ACETYLCOLINE (ACh), ACETYLCHOLINESTERASE (AChE), AND CYCLIC AMP (cAMP).** C. Woody, H. Sakai*, M. Sakai*, and E. Gruen*. Departments of Anatomy & Psychiatry, Brain Research Institute, Mental Retardation Research Center, UCLA Medical Center, Los Angeles, CA 90024.

This study examines the effects of extracellular application of acetylcholine (ACh) and cycloheximide (cHx) on the excitatory responses to acetylcholine (ACh) in the neocortical neurons of the rat. The researchers observed that ACh and cHx produced no effect on neostabilized cyclic AMP levels in these same neurons. The potential of NE-induced accumulation of cyclic GMP by GABA was also dependent upon the concentration of the NE used. GABA evoked a potentiation of cyclic AMP accumulation at the same concentrations of NE that are not present in artificial seawater with lithium (Li-seawater) on the excitatory responses of morphologically identified mammalian, neocortical neurons. This suggests that there are common ionophores for each class of ionic response (Swann and Carpenter, Nature 236: 751, 1973). If the ionophores mediating the increases to several neurotransmitters are attributable to the pharmacologic action of these agents.

The observation that lithium affects the fast excitatory responses to dopamine and serotonin in a similar manner supports the hypothesis that they are mediated by the same ionophore. Since lithium acts differently on the slow excitatory response to serotonin, a different ionophore may be involved.

**2048**


This study investigated the effects of lithium on excitatory responses to serotonin and dopamine. Preliminary results suggest that the slow excitatory response to serotonin is reduced in amplitude and prolonged by 50% Li-seawater. There is a further reduction in amplitude in 100% Li-seawater, but the response was not completely abolished. The reduction in amplitude was partially reversible. The fast conductance increases due to serotonin and dopamine were also reduced in amplitude at both lithium concentrations. These responses were not reversed by lithium. Normal seawater partially reversed the reduction in amplitude of the responses to both neurotransmitters.

The observation that lithium affects the fast excitatory responses to dopamine and serotonin in a similar manner supports the hypothesis that they are mediated by the same ionophore. Since lithium acts differently on the slow excitatory response to serotonin, a different ionophore may be involved.
BICUCULLINE DISPLACES 3H-MUSCIMOL AND NA-INDEPENDENT 3H-GABA BINDING IN CULTURED MAMMALIAN SPINAL CORD AND FOREBRAIN NEURONS. A.R. Young and R.L. Macdonald. Department of Neurology, University of Colorado Medical Center, Ann Arbor, MI 48109.

The neutral amino acid γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system and plays a wide distribution in brain as well as in spinal cord. Electrophysiological and neuropharmacological studies using varied in vitro preparations have suggested that many antagonist and anticonvulsants drugs interact with GABAergic systems to produce their pharmacological effects. Of particular interest is the relationship between convulsonal-induced paradoxical activity and antagonism of GABA-mediated inhibition. To approach the mechanism of action of these agents, we have used primary dissociated neuron cultures derived from fetal rat brain and spinal cord and have investigated the action of convulant and anticonvulsant drugs on the uptake, release and binding of putative amino acid neurotransmitters. Such studies permit direct comparison of neurochemical and electrophysiological data using the same mammalian preparation. We report here that the convulant alkaloid bucculline displaced sodium independent GABA-binding in both brain and spinal cord cultures but that the ED₅₀ for GABA-displacement was substantially different in the two culture systems. Spinal cords and forebrains were removed from 13-14 or 15-15.5 day old fetal mice respectively, mechanically triturated and plated in 35 mm collagen-coated culture dishes. Spinal cords were plated at 1/2 cord/dish while forebrains were plated at 1/3 hemisphere/dish. At three to eight weeks, the cultures were suspended in 50 mM tria-citrate buffer at room temperature. Following addition of Triton, homogenization and incubation for 30 min at 37°C the membranes were centrifuged at 48,000g for 20 minutes. The membranes were then twice resuspended in buffer, homogenized and centrifuged. GABA receptors were measured by 3H-muscimol binding or Na-independent 3H-GABA binding. Binding was measured in the presence or absence of GABA, thiadiazole acetic acid and muscimol but not by glycine or 2,4-diaminobutyric acid (a GABA uptake inhibitor). Bucculline displacement of GABA-binding had an ED₅₀ of 5-7µM in brain cultures. In spinal cord, the ED₅₀ for bucculline was more variable but appeared to be between 20 and 100µM. To confirm these data, we performed similar experiments on tritomized adult rat brain and spinal cord membranes and report that the ED₅₀ for bucculline displacement of GABA-binding was 5 and 40µM respectively. Thus these studies demonstrate that the convulant alkaloid bucculline has different effects on brain and spinal cord receptors both in vitro and in vivo. These differences determined biochemically in vitro have been compared to electrophysiological studies of bucculline-induced paroxysmal discharges using the same preparation (see abstract of Macdonald, Young and Nowak).


Guanine nucleotides and monovalent cations decrease the apparent affinities of agonists for striatal dopamine receptors. In the presence of GTP, the apparent affinity of H-APOMORPHINE bound as well as the potency of agonists measured by inhibition of H-spiroperidol binding to rat striatal membranes were decreased. These GTP-dependent decreases in the apparent affinities of agonists for the dopamine receptor are due to an increase in the rate of dissociation of agonists from the receptor. At 15°C the half-time of dissociation of the partial agonist H-APOMORPHINE from dopamine receptors in striatal membranes was approximately 25 minutes. The addition of 0.3 mM GTP to the incubation medium caused a 3-4 fold increase in the rate of H-APOMORPHINE dissociation. At 15°C the halftime of dissociation was 2 minutes in the presence of GTP. Thus, in the presence of GTP little specific H-APOMORPHINE binding was measurable at 37°C. The presence of monovalent cations (30-150 mM) caused a 2-fold increase in the ability of agonists or partial agonists to inhibit H-spiroperidol binding. The mechanism by which monovalent cations produce their effects has not yet been established. It is interesting to note that the agonist-specific effects of GTP were observed either in the presence or absence of Na. Similar effects of monovalent cations were seen in studies of agonist inhibition of H-QNB binding to muscarinic cholinergic receptors in rat caudate. GTP, in the presence or absence of monovalent cations, however, had no effect on either the affinity of H-QNB or the inhibition of H-QNB binding by muscarinic agonists or antagonists. Neither purine nucleotides nor monovalent cations had any effect on the binding properties of dopaminergic or muscarinic cholinergic receptor antagonists. On the other hand, divalent cations increased the affinities of agonists and decreased the affinities of antagonists for both dopaminergic and muscarinic cholinergic receptors. The results of this study together with those from other laboratories support the hypothesis that GTP affects agonist binding to receptors which are linked to a benzylic cyclase. Since monovalent cation effects are seen with receptors that are and are not sensitive to GTP, these results suggest that the mechanism by which GTP and monovalent cations produce agonist-specific effects is not the same.

This work was supported by the USPHS (NS 09199) and by an NIH fellowship (NS 05970).
PAIN

The analgesic action of morphine is in part mediated through the periaqueductal gray area (PAG). The analgesic action of morphine is produced by injection of glutamate into the PAG, rats were made dependent on morphine by implanting with two pellets, each containing 75 mg of morphine. After 24 hours, the animals were anesthetized with urethane and single cells were recorded from the PAG. Glutamate (1µl, 50mM) was injected into the PAG with a microsyringe. The analgesic effect of glutamate was measured by recording the EMG from the flexor muscle of a hind leg ligated by noxious heat. The result shows that glutamate injected into the PAG of morphine tolerant animals does not produce analgesia. However, if the animal is pretreated with naloxone and then glutamate is injected into the PAG, it does produce analgesia. In morphine tolerant animals, injection of glutamate into the PAG produces a slight increase in the firing rate of these cells. The injection of glutamate into the PAG produces a significant increase in the firing rate of these cells. In order to determine the effect of glutamate on these cells, animals were implanted with an indwelling cannula and after recovery, tail flick latency was measured before and after injection of glutamate (1µl, 50mM). Glutamate injection did not produce analgesia but after injection of glutamate, the tail flick latency was measured. After this measurement, the animal was implanted with two more glutamate pellets. 72 hours later, glutamate injection did not produce analgesia. The results suggest that chronic morphine treatment decreases the stimulus induced release of neurotransmitter involved in the interaction between the PAG and the NRM.

PAIN

2051 DEAFFERENTATION: EFFECTS OF TOOTH Pulp EXTRAPATION ON TRIGEMINAL BRAINSTEM NEURONS. J.G. Bell, J.O. Drostovsky, J.W. Hu and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Canada, M5G 1X8

Recent studies have shown that deafferentation can lead to marked structural and physiological changes in central neuronal organization that are related to the survival of denervated axons and associated sensory loss. Since it has recently been reported that partial tooth pulp extrapation results in trans-nociceptive changes in neurons of the trigeminal brainstem sensory nuclear complex, we wished to determine if the functional properties of V brainstem neurons change accordingly. All sections were then carefully scanned and the location of retrogradely labeled cells charted. The predominant descending input could be explored. We have initially investigated the effect of aseptic removal of the coronal pulp of the ipsilateral mandibular canine and premolar and solar teeth on the functional properties of single neurons recorded in the V spinal tract nucleus 13-28 days following the pulp extrapation. These recordings were made in adult chloralose-anesthetized rats and were primarily, although not exclusively, directed at neurons in subnucleus oralis. Neuronal properties in the ipsilateral nucleus were compared with those examined in the contralateral nucleus of the same animal and in the nucleus of other animals which were not subjected to pulp extrapation. These properties included adequate stimulus, receptive field size, von Frey hair threshold, somatotopic pattern, spontaneous activity, inhibitory influences from afferent or nucleus raphe magnus sources, and short-latency excitatory input arising from bilateral electrical stimulation of the remaining canine and premolar pulps, infraorbital and superior laryngeal nerves, and facial skin. Some of these functional properties were modified in neurons ipsilateral to the pulp extrapation. The most obvious changes included an increase in spontaneous activity that often had an erratic bursting character, an increase in receptive field size and number of excitatory inputs, and a partial disruption of the normal "inverted-face" somatotopic pattern of the nucleus; no obvious difference was noted in the inhibitory influences studied, or in the properties of V primary afferents in the nucleus. Most of these changes occurred in neurons located in the dorsal one- to two-thirds of the nucleus, and indicate that considerable functional changes may occur in V brainstem neurons as a result of a partial loss of sensory input from the tooth pulp.


Pain threshold elevations in rats occur following acute exposure to a wide range of severe environmental stressors. The anti-nociceptive effects of several stressors appear to act independently of the endogenous opioid pain-inhibitory system, but are similar to the effects of histaminergic, non-opioid nociceptive inhibitory function. The present study examined the role of discrete hypothalamic mechanisms in mediating stress-induced analgesia in the rat. The anti-nociceptive effects of stress on nociceptive responses were studied using the tail flick assay and the antinociceptive effects of cold-water swim analgesia. In the first experiment, six rats were exposed neonatally to monosodium glutamate (MSG-2mg/kg) while six littersmate controls received placebo. In the second experiment, 300 rats were tested for basal reactivity to foot shock while as well as their analgesic responses to cold-water swims, morphine, food-deprivation and 2-deoxy-d-glucose. MSG treatment altered shock detection but not basal nociceptive thresholds, while selective analgesic deficits were observed following food deprivation and 2-deoxy-d-glucose. In addition to the known deficits in arcuate dopamine and choline acetyltransferase, subsequent immunocytochemistry revealed that there was a marked, loss of arcuate cells containing ACTH and V-1B-glycoprotein in the hypothalamus, and absence of vasopressin were examined for basal reactivity to cold-water swims. Normal analgesia response to cold-water swim analgesia was not significantly affected by acute morphine treatment. Partial hypothalamic lesions did not significantly affect responses to cold-water swim analgesia. While peripheral administration of the vasopressin analogue DDAVP reversed the diabetes insipidus and increased basal nociceptive sensitivity, it failed to alter analgesic deficits in cold-water swim analgesia. In DDAVP-treated rats, both opiate and non-opiate analgesia were observed following food deprivation and 2-deoxy-d-glucose. The results suggest that chronic morphine treatment decreases the stimulus induced release of neurotransmitter involved in the interaction between the PAG and the NRM.
PAIN


The ventrolateral periaqueductal gray (PAG) has been implicated in the mediation of opioid and stimulation-produced analgesia. The present study examined whether this region also subserves the antinociceptive properties of stress and non-narcotic psychotropic drugs. Tail-flick latencies were measured with noxious stimuli presented to terminate its pain. It is suggested that if the CM-PF is an integrating area for pain, entrapment of the firing patterns of neurons by morphine may precede release of impulses encumbering painful stimuli and thus inhibit the integration of such stimuli. The appearance of invariant patterns may be related to morphine mania or the euphorogenic properties of the drug.


Single unit activity was recorded chronically in the centromedial-parafascicular (CM-PF) complex of the feline thalamus using fine wire semimicroelectrode bundles. Animals were awake and freely moving.

Morphine was injected (1.0 mg/kg IM) and produced the classic signs of feline morphine mania including desynchronised electrocorticogram, mydriasis and hyperreactivity to environmental stimuli. Analysis of neuronal interspike intervals was performed with an inequality testing pattern detection technique which made statements about consecutive interspike intervals; an interval was either longer (-) or shorter (+) than the previous interval. Occurrences of patterns composed of 3 through 6 signs were tabulated and empirical frequency distributions constructed. Empirical distributions of patterns were compared with those expected from a theoretical model based on independence of sequential signs, using the chi-square test (see Bruce and Marczyinski Brain Res. 125; 65, 1977; Marczyinski and Burns Neurosci. Abst. 1978). Neurons showed an increase in patterned activity throughout the course of action of morphine (3-4 hours). Ensembles of patterns developed which were invariant during the morphine effect. In the same neurons during other behavioral states such as slow wave sleep, quiet wakefulness and bar pressing for milk, different patterns developed usually with greater variability than those observed following morphine. The onset of the patterns occurred gradually during the first 40-60 min following injection, remained stable for 1-2 hours, then slowly declined. Naloxone (0.015 mg/kg) was injected 60 min after morphine in two animals. Within 3 min, the patterns so prevalent with morphine either decreased or disappeared entirely, along with the signs of morphine mania. They were absent for 15-20 min, then the same pattern ensembles reappeared in concert with the reappearance of signs of morphine mania and persisted for another 1-2 hours. All cells recorded also showed dramatic increases in their firing rates during the action of morphine.

Injections of the opiate antagonist naloxone (0.015 mg/kg) were made 1-30 min after another injection with much shorter duration of action (30-45 min). Smaller doses of morphine (0.3 mg/kg) and fentanyl (0.00375 mg/kg) did not show these patterns.

It is suggested that if the CM-PF is an integrating area for pain, the entrapment of the firing patterns of neurons by morphine may precede release of impulses encumbering painful stimuli and thus inhibit the integration of such stimuli. The appearance of invariant patterns may be related to morphine mania or the euphorogenic properties of the drug.


Rats were implanted with two monopolar stimulating electrodes. One was directed at the lateral hypothalamus (LH) and the other at medial nucleus reticularis gigantocellularis (NGC). These rats were found to leverpress for 3-second trains of LH stimulation during continuous NGC stimulation. Secondly, the LH-currents used in this experiment were too low to support self-stimulation in the absence of continuous NGC stimulation, we concluded that pressing LH during the extinction phase of the NGC-aversion. However, in order to control for the alternative explanation that the LH-trains had been made rewarding by association with the NGC stimulation, another test was conducted. By using raised currents, rates of pressing for LH-trains in the absence of NGC stimulation were equated with those obtained by using the previous, lower, currents in the presence of continuous NGC stimulation. It was found that gastric-loading depressed responding for LH-trains in the absence but not in the presence of continuous NGC stimulation. Thus, it would seem that leverpressing for LH-trains during NGC stimulation is maintained by aversion-ameliorating effects of LH stimulation which are not identical with the reward effect.

Rates of leverpressing for LH-trains during continuous NGC stimulation are greatest when the two trains are phased so that each pulse in the LGP NGC train is preceded by a pulse to LH at an interval of 1.5 or 10-15 msec. These two peaks of inhibition in the leverpressing function suggest that this aversion-ameliorating effect is due to a bimodal gating mechanism. A second experiment was conducted in which the reverse of the above experimental procedure was followed. That is, rats leverpressed for 3-second periods of escape from NGC stimulation during continuous stimulation of LH. The continuous LH stimulation reduced rates of leverpressing for LH when the escape from NGC stimulation was greatest when each pulse in the 25 pps NGC train was preceded by a pulse to LH at an interval of 1.5-5 or 10-15 msec. These two peaks of inhibition are produced by intermittent stimulation of the NGC-aversion. By contrast, caudal PAG lesions increased LH responses to CDP, 2-DG. These data indicate that the PAG, while apparently important for both opiate and stimulation-produced analgesia, plays no role in the antinociceptive properties of stress or psychotropic drugs.
To determine if these inhibitory mechanisms are endogenous opiates and serotonin—have differential effects on spinal neuronal inhibition from PAG and LRF. Lumbar dorsal horn neurons with A- and C-fiber input from the limbic cutaneous nerves were recorded in lightly anesthetized cats. Units responded to noxious radiant heat stimuli (50°C, 10 sec) applied at 3 min intervals either without or during stimulation of PAG or LRF. Inhibition exhibited a latency ratio: heat-evoked discharge during midbrain stimulation/discharge in the absence of midbrain stimulation.

If descending inhibition is mediated by endogenous opiates, blockade of opiate receptors with the opiate antagonist naloxone should reduce inhibition. Naloxone (1-3 mg/kg i.v.) had no effect on inhibition of spinal neuronal heat-evoked responses by PAG stimulation (Neurosci. Lett. 11:323, 1979). However, naloxone partially reduced inhibition produced by stimulation of one (of 8 units studied to date. Inhibition was never completely blocked by naloxone.

Blockade of presynaptic receptor by the serotonin antagonist methysergide (0.5 mg i.v.) greatly reduced or abolished inhibition from PAG in each of 5 units. Higher doses partially or partially reduced the effect of LRF in 2 of 4 units. In rats pretreated with the serotonin synthesis inhibitor para-chlorophenylalanine (PCPA, 500 mg/kg, i.p.) PAG was significantly reduced while inhibition from LRF was enhanced.

These results indicate that serotonin mediates inhibition from PAG but not LRF. Endogenous opiates may play a modulatory role in descending inhibition from LRF which, however, is mediated by some other neurotransmitter. The role of catecholamines in descending inhibition from midbrain is currently under study.

Recent research has delineated the existence of intrinsic pain inhibitory mechanisms which may be activated by acute stress, lesion-induced hypermotony or intense fear. These analgesic states have been termed autoanalgesia in that the antinociception is locally-regulated and therefore a result of the intrinsic neuronal activity of endogenously-synthesized molecules. Although initial observations suggested that autoanalgesia may result from activation of an endorphin system within the CNS, additional investigations using endorphin modulating agents failed to demonstrate the ineffectiveness of naloxone in reversing the anti-nociceptive effect as assessed by tail-flick tests. Since recent research (Koss et al., Neuropharmacology 18: 295, 1979) has indicated that yohimbine may block tachyphylactic inhibition in these animals, we investigated the efficacy of this drug in antagonizing autoanalgesia and morphine analgesia.

Antinociception was assessed using the rat tail-flick procedure with basal latencies of 2-3 sec, a cut-off criterion of 8 sec being maintained. In the first experiment (n=20), the effects of yohimbine HCl (5 mg/kg; i.p.) on basal tail-flick latencies was assessed. In the second experiment, the effects of yohimbine (5 mg/kg) on analgesia acutely elicited by footshock (1.0 mA; 15 sec.) or by the classical conditioning of fear to the tail-flick procedure (Chance et al., Brain Res. 141:371, 1978) was investigated. The last experiment (n=27) examined the effect of yohimbine (5 mg/kg) on analgesia elicited by morphine (8 mg/kg; s.c.). Yohimbine decreased basal tail-flick latencies by 47% in the first injection, with this hyperalgesic response lasting for at least 3 hr. Analgesia elicited by acute footshock was reduced to control levels following administration of yohimbine. As in previous research, classical fear conditioning elicited analgesia which increased to an asymptote by day 5 (6.2 ± 2.6 sec). Yohimbine dose-dependently reduced this antinociceptive effect to 1.7 sec by day 6. Morphine analgesia was also reduced 52% by yohimbine, 15 min after administration of the opiate. This reduction, however, did not increase 32% at 30 min after administration of the opiate. These observations demonstrate the effectiveness of yohimbine and suggest that this antinociception is due to removal of inhibitory influences descending to the spinal cord by means of a 27-gauge needle which is connected to a microinjection device. Electrical activation of NRGC was induced via a stereotaxically placed bipolar concentric electrode.

Bilateral microinjection of morphine to NRGC, at a dose of 6.5 mg/kg, elicited a small, though significant depression of the JOR. Significant inhibition of the evoked JOR, however, result when a dose of morphine at 10 mg/kg was introduced, sustaining for 30-60 min. Such suppression was reversed by naloxone, either administered systemically (1 mg/kg) or microinjected bilaterally into the NRGC (1 mg/kg).

The effect of NRGC stimulation on dental pulp evoked trigeminal responses was studied using the conditioning-test technique. NRGC activation immediately elevated analgesia. 100% suppression of the intradentally evoked orals potential in all animals within 5-10 msec after the beginning of the reticular activation. This was followed by a slow and continued reduction of decline in inhibition. Complete restoration of the control amplitude in most cases did not take place until 500-800 msec following the reticular activation. The effect has been observed on JOR.

The present study demonstrated that the NRGC is a common neural substrate for morphine and focal stimulation suppression of dentin, LRF. Parallel investigations in this laboratory suggest that upon activation by the opiate or electrically, neurons in NRGC may prevent the transmission of nociceptive signals from the dentin to carryout a de-polarization of the pulpal afferents, thus allowing a process that may also involve the opiate receptors and enkephalins.

Recent evidence shows that systemic treatment with antipeptidase (D-leucine and D-phenylalanine) induces hypalgesia (pain reduction) in man and mice and also causes no addiction. These antipeptidases are postulated to act by protecting endorphins from peptidease destruction, since the hypalgesia they produced was naloxone-reversible. To further test the anti- peptidase-endorphinergic hypothesis we compared antipeptidase effects in three related strains of mice. One strain (C57BL) is low in opiate receptors and exhibits poor electroacupuncture and morphine analgesia; another strain (B6D2F1) has abnormally high levels of pituitary B-endorphin. A third strain (B6AF1) is used as control as it exhibits normal electroacupuncture hypalgesia. The antipeptidases increase electroacupuncture hypalgesia in order of Ob/Ob > B6D2F1 > B6AF1. This correlates with the differences in endorphinergic systems in these 3 strains of mice. In addition, these antipeptidases increase electroacupuncture hypalgesia in B6AF1 mice. The combination of antipeptidase and electroacupuncture may provide a non-addictive method for clinical pain treatment.
The endorphins produce morphine-like actions on pain systems, and these actions are blocked by the specific narcotic antagonists naloxone and naltrexone. Although the endorphin systems are characterized, few practical attempts have been made to manipulate this system, so as to produce analgesia. We were intrigued by the potential analgesic actions of another neurotransmitter system, where chronic administration of the antagonist results in a paradoxical potentiation of the agonist's actions. We have now tested the efficacy of potentiation from morphine-endorphin systems. Studies were performed in both the Hot Plate test and the tail-flick test. Animals were treated with saline and tested for analgesia develop an apparent analgesia over time. This effect is most likely related to the stress-induced analgesia in response to the thermal stimulus used in testing. In contrast, animals receiving naloxone under similar conditions failed to develop analgesia. This data would suggest that the stress-induced analgesia is most likely mediated via endorphin systems as would be consistent with its reversibility by the specific narcotic antagonist naltrexone. The data also demonstrate that chronic naloxone produces a marked potentiation of morphine-induced analgesia. This phenomenon may be explained by supposing either that naloxone has produced a sensitization of opiate receptors, either by increasing their numbers or affinity, or by altering morphine's pharmacokinetics. Thus prior treatment of saline treated animals may lead to increased efficacy, and thus a decreased dosage of opiates used in clinical situations.

**Electrophysiological Evidence for a Direct Projection from the Periaqueductal Gray to Nucleus Raphe Magnus in the Cat and Rat.** Jonathan O. Dostrovsky and Yasmin Shah*, Department of Physiology, NIDR, NIH, Bethesda, MD 20205.

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**STRESS AND ANTAGONIST POTENTIATION OF OPIATE ANALGESIA.** J. E. Cowen, R. L. Borison, R. S. Nadvala* and B. D. Diamond, Mount Sinai Hospital, Dept. of Anesthesia, Chicago, IL 60602.

The influences of adrenergic and 5-hydroxytryptaminergic (5HT) systems on morphine analgesia (MA) have been explored in many psychopharmacological studies (cf. Takemori, Ann NY Acad Sci., 281: 262, 1976). Unfortunately, the results are highly variable, and a consensus on 5HT and adrenergic mechanisms has been difficult to reach. An important step towards the ability to be the pain test. We have been systematically examining the effects of adrenergic and 5HT antagonist agents on pain as measured in rats by hot plate, Hot Plate and Formalin. These tests differ in the type of noxious stimulation and in the behavioral responses from which pain is inferred. Each test reveals a unique psychopharmacological profile.

In the Tail-Flick test, analgesia was produced and MA enhanced by L-tryptophan (>200 mg/kg), a 5HT precursor. MA was reduced by the inhibitor proopiomelanocortin (1 mEq/kg) but not by high (74 mg/kg) doses. In this test, α-adrenergic agents and L-tryptophan had little or no effect in this form. In the Formalin test, the α-agonist clonidine (0.03 mg/kg) and the β-agonist isoproterenol (>0.5 mg/kg) were analgesic and synergized MA. Yohimbine and propranolol (both >1 mg/kg) were somewhat analgesic alone and appeared to add to MA. When combined, yohimbine (1 mg/kg) and propranolol (1 mg/kg) antagonized MA by >2 mg/kg, L-tryptophan (>200 mg/kg), p-chloroamphetamine (PCA, 10 mg/kg), and p-chlorophenylalanine (PCPA, 150 mg/kg) enhanced MA while PCA (20 mg/kg) antagonized MA in this test. PCA and PCPA deplete 5HT.

It appears that different pain tests reveal different underlying neural substrates, so that a given antagonist will have different effects on the test insufficient to characterize pain in general. Both the type of noxious stimulation—heat or subcutaneous formalin—and the rate of recovery after treatment and site of application contribute to the observed variability. There appear to be multiple adrenergic and 5HT systems—some cooperatively related, some competitive—that influence pain and analgesia.

Supported by Grant A-7891 to RM from the Canadian NRC and by an MRA from NHDCS to SD. Thanks to S. Gutman and F. Boucher.

**Adrenergic and 5-Hydroxytryptaminergic Influences on Morphine Analgesia: Assessment by Three Pain Tests.** R. S. Dennis and B. D. Diamond, Dept. Psychol., McGill University, Montreal, Quebec, Canada H3A 1B1

The influences of adrenergic and 5-hydroxytryptaminergic (5HT) systems on morphine analgesia (MA) have been explored in many psychopharmacological studies (cf. Takemori, Ann NY Acad Sci., 281: 262, 1976). Unfortunately, the results are highly variable, and a consensus on 5HT and adrenergic mechanisms has been difficult to reach. An important step towards the ability to be the pain test. We have been systematically examining the effects of adrenergic and 5HT antagonist agents on pain as measured in rats by hot plate, Hot Plate and Formalin. These tests differ in the type of noxious stimulation and in the behavioral responses from which pain is inferred. Each test reveals a unique psychopharmacological profile.

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2067 APPARENT SAFETY OF HIGH ELECTROANALGESIA CURRENT APPLIED TO INTACT TEETH. R. Wayne Fields, Patrick J. Reynolds, Robert P. O'Connel* and Richard B. Tuck. School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

We have demonstrated the effectiveness of electric current, applied to teeth in vivo, in lessening the amplitude of trigeminal field potentials (Fields, et al., Exp. Neurol. 47:229-236, 1975) or responsiveness of identified primary afferents (Fields, et al., Exp. Neurol., 53:386-396, 1976) to electrical test stimuli applied to the teeth. Presumably similar currents have been administered using a remote cathode with the anode applied to the exposed dentin of the tooth in question. Direct currents applied to vital elements of tooth pulp (Fields, et al., Arch. Oral Biol. in press).

When much higher currents, to 1000µA, are used, long lasting hyperexcitability follows administration of direct current, while the effects of pulsatile current appear to be reversible (Fields, et al., Exp. Neurol., 47:229-236, 1975) or responsiveness of identified primary afferents (Fields, et al., Exp. Neurol., 53:386-396, 1976) to electrical test stimuli applied to the teeth. Presumably similar currents have been administered using a remote cathode with the anode applied to the exposed dentin of the tooth in question. Direct currents applied to vital elements of tooth pulp (Fields, et al., Arch. Oral Biol. in press).

We have shown that pulsatile currents with peak levels as low as 70µA can be used as effective direct current to lower after-emergent excitability (Fields, et al., Arch. Oral Biol. in press). When much higher currents, to 1000µA, are used, long lasting hypoexcitability follows administration of direct current, while the effects of pulsatile current appear to be reversible (Fields, et al., Arch. Oral Biol. in press). In order to establish the clinical safety of electroanalgesia (EA) current we felt it was essential to examine tooth pulp histologically at intervals following administration of current. Such observation requires that the teeth remain intact to insure that pulpal changes are not secondary to invasion of the dentin.

We utilized both dc and ac (rectangular pulses at 1000µA, 10 duty cycle, capacitively coupled) at peak levels spanning the range known to be effective in lowering after-emergent excitability and including the potentially damaging value of 1000µA. The anode was a saline soaked cotton wick in contact with a platinum wire, all encompassed in a length of sialic tubing pushed firmly onto the crown of the intact tooth and sealed in place with wax. The test electrodes were a dentistry grounding pad on a hindlimb. Acute current administration was done under ethane anesthesia, and teeth were harvested on days 2, 8, 21 and 60.

In no case, either with direct current or alternating current at the same peak levels of 1000µA were histologically significant pulp damage apparent. The lack of expected damage with high direct current may be related to diffusion of the current by the saliva. Our results indicate that even at low levels, current applied to intact enamel effectively lowers excitability.

(Submitted by NIH Grant DE 04281)


The influence of the narcotic antagonist naloxone and placebo on the experience of postoperative pain was examined in 36 patients following extraction of one or more mandibular third molars. Extractions were performed with a short duration local anesthetic (2% lidocaine without epinephrine) and without the use of intravenous or inhalation sedatives. Visual analog (VAS) and verbal descriptor (VDS) scaling procedures were used to assess responses related to the sensory intensity, unpleasantness and painfulness of the postsurgical pain for 2 hours beginning 1 hour after the initiation of local anesthesia. Two groups of 12 subjects received double-blind intravenous injections of either 10 mg naloxone or saline vehicle (placebo) 2 hrs after injection of local anesthesia. A third group received no treatment. Both the VAS and VDS scales showed increased responses following no treatment and decreased responses following both. These differences were statistically significant for both the VAS (order X drug interaction, F (1,22) = 5.29, p<0.05) and VDS (order X drug interaction, F (1,22) = 6.38, p<0.05) scales of unpleasantness. VAS and VDS scales of sensory intensity and painfulness showed no statistically significant effects. VAS scales did not significantly differentiate between naloxone and placebo on any dimension. The VDS scales, however, showed a significant elevation in unpleasantness responses after naloxone in comparison to placebo (order X drug interaction, F (1,22) = 43, p<0.05). This effect was not significant for VDS scales of sensory intensity or painfulness. This experiment documents a significant difference in the unpleasantness of postsurgical pain following administration of the opiate antagonist naloxone and placebo, and an inability to explain this difference by reversal of sedative agents used during surgery. The results suggest that naloxone increases the unpleasantness associated with postsurgical pain with respect to the placebo. These opiopiate-like substances present after surgery or after the administration of a placebo. The superior sensitivity of the unpleasantness scales compared with those of painfulness probably results from factor analyses of verbal pain responses and further stresses the importance of multidimensional assessment of pain experience.


Naive rats subjected to one of a number of different stressors display temporary analgesia which may last as long as 2 hours (after treatment of the stressor). We have reported previously that this phenomenon, generally called stress-induced analgesia (SA), is greatly attenuated by hypophysectomy (Bodnar, R.J. et al, Pain Abst. 1:262, 1978; Physiol. Behav. 23: 1979). Similar results, in agreement with ours, have been obtained by others (Pert, A., 10th Annual Winter Conference on Brain Research, 1978; Amir,S. Abstracts, 1:262, 1978; Physiol. Behav. 23: 1979). We now report that this drug-induced attenuation of SA is dosedependent largely on the integrity of the anterior lobe of the pituitary. For a period of 2 weeks each, Group I consisted of rats in which the whole pituitary had been surgically removed; Group II had the anterior pituitary remaining intact; and Group III consisted of sham operated controls. For a period of 2 weeks each, post-operatively and throughout the experimental test period, extractions were performed with a short duration local anesthetic (2% lidocaine without epinephrine) and without the use of intravenous or inhalation sedatives. Visual analog (VAS) and verbal descriptor (VDS) scaling procedures were used to assess responses related to the sensory intensity, unpleasantness and painfulness of the postsurgical pain.

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(Submitted by NIH Grant DE 04281)
Electrophysiological examination of primate spinoreticular tract neurons. Lawrence H. Haber, Bart D. Moore* and William D. Willis. Marine Biomedical Institute and Depts. of Anatomy and Physiology and Ophthamology, Univ. Texas Medical Branch, Galveston, TX 77550.

Various lines of evidence suggest that cells in the nucleus reticularis gigantocellularis (NRC) may serve as a bulbar relay in a spino-reticulothalamic nociceptive pathway. The present work was undertaken to elucidate the response properties of spinal neurons projecting to this area of the caudal brain stem.

The experiments were carried out on young rhesus monkeys (Macaca fascicularis). Twenty-nine spinoreticular (SR) neurons in the lumbar (10 cells) and cervical (19 cells) enlargements were identified by antidromic activation from the region of the ipsilateral (9 cells) or contralateral (20 cells) NRC. Three of the cells could also be antidromically activated from the ventro-posterior lateral nucleus of the contralateral thalamus. The threshold stimulus strength to produce an antidromic response varied from 20-300 µA (less than 100 µA in a majority of cases). Conduction velocities of SR neurons ranged from 9-51 m/s (mean = 24.0 m/s ± 9.6). Fifteen of 29 SR neurons could be excited by natural stimulation of one or more limbs. Of these, 1 cell was activated by tactile stimulation, 3 cells were excited by tactile stimulus but their discharge was enhanced still more by noxious mechanical stimulation (i.e., these cells had a wide dynamic range). Nine cells were excited only by electrical stimulation of the skin and 2 cells were excited exclusively by stimulation of the deep tissues. SR cells having peripheral receptive fields were laterated in nearly all regions of the spinal gray matter, whereas SR neurons unresponsive to peripheral stimuli were located primarily in laminae VII and VIII. The discharge of SR cells could be facilitated or in fewer cases inhibited by brain stem stimulation.

The implication of these experiments is that spinal neurons which send their axons to the brain stem may be important for the transmission of sensory information necessary for some aspects of pain perception and response. The work was supported by a research grant (NS 09743) and by a postdoctoral fellowship to L.H. Haber (NS 05087) from the National Institutes of Health.

Supported by the Canadian MRC and the Quebec MRC.


Normal grooming responses of the cat consist of licking, nibbling, wiping the face and ears with the forepaw and scratching the head, neck and shoulders with the hindpaw. These responses are inhibited by systemic or local administration of local anesthetics or narcotics. After bilateral neodestruction or after bilateral removal of the frontal poles including the primary somatosensory area the cat becomes incontinent and grooming responses gradually reappear and by 30 to 50 days post-op have become markedly exaggerated demonstrating the CNS nature of the deficit. Gross grooming may become so marked that the animal cannot stand nor walk being consumed by licking. The reflex to grooming in the decorticate cat are essentially the same as in the normal animal. The grooming movements, however, have periorbital sign and are frequently ineffectual since they are often misdirected. The type of response elicited is also related to the intensity of the stimulus and a lesser extent to the modality of the stimulus.

Nociceptive responses such as the flexion reflex, flinching or a limb, tail lashing, grooming, and spitting can be completely inhibited by eliciting the licking response. This inhibition persists from 15 seconds to 2 minutes after cessation of reflexly induced grooming. The longer inhibition is related to a longer period of induced grooming. The nociceptive response then rebounds with even greater intensity but can be inhibited again by eliciting grooming. To determine if the inhibition of the nociceptive response by licking was mediated by endogenous morphine-like substances, we treated the cat with Naloxone (0.4-4 mg/kg body weight) which blocks the receptor. The treatment did not alter the grooming nor its inhibition of the nociceptive responses. We conclude that excessive grooming and the dominance of the grooming response over nociceptive responses in the decorticate cat is not due to endogenous opiate release. Possible mechanisms will be discussed.

Supported by the Canadian MRC and the Quebec MRC.
PITUITARY-ADRENAL ACTIVITY ASSOCIATED WITH CENTRAL PAIN FOLLOWING COMPLETE FORELIMB DEAFFERENTATIONS IN RATS. J. P. Heynich,* N. Levitt and A. Brodish.* Dept. of Physiol. and Pharm., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

In the present study behavioral and hyperreactivity to air puffs directed at lumbar body dermatomes. Control rats were not self-mutilating and consisted of: 2 rats with extensive dorsal rhizotomies; 1 rat with 2 dorsal roots cut bilaterally; and 1 rat with laminecency and dural incision, but no rhizotomies. Deafferented self-mutilating rats were compared to the control rats with regard to basal plasma corticosterone (B) levels, as an index of pituitary-adrenal activity. Blood samples were taken (0800-1000h) by jugular venepuncture under ether at various pre- and postoperative intervals. Dramatic elevations in plasma B levels corresponded to the day of onset of self-mutilation but no rhizotomies. Deafferented self-mutilating rats were identified by its antidromic responses to stimulation at the presumed central pain. In support of the latter it was noted that the elevated B levels were simply a response to peripheral somatic tissue damage with any of the motor behaviors accompanying panel release. The burst of neural activity was temporally related to panel release, but it was not reliably correlated with any of the motor behaviors accompanying panel release. Finally, the burst of discharges was maximal and eventually disappeared when reinforcement was withdrawn during experimenter-presented trials. These findings indicate that some neurons in DHM have a functional relationship with noxious peripheral stimuli. For the moment, the question of whether neurons in DHM which project to contralateral trigeminal mesencephalic nuclei are involved in the projection. We have tested for evidence for the release of either peptide after activation of nociceptive afferents. By superfusing the mammalian spinal cord with noxious or innocuous orofacial stimuli. A neuron projecting to the ipsilateral oralis and/or contralateral posterior thalamus was identified by its antidromic responses to stimulation at these sites. Effects of conditioning stimulation of PGM and NRM were tested on both orthodromically excited and antidromically evoked activity. A decrease in orthodromic responsiveness reflected inhibition of the caudalis neurons; increase in antidromic excitability was an indirect measure of presynaptic depolarization of the axonal endings in oralis of the caudalis neurons. An increase in antidromic excitability was an indirect measure of presynaptic depolarization of the axonal endings in oralis of the caudalis neurons. An increase in antidromic excitability was an indirect measure of presynaptic depolarization of the axonal endings in oralis of the caudalis neurons. An increase in antidromic excitability was an indirect measure of presynaptic depolarization of the axonal endings in oralis of the caudalis neurons. An increase in antidromic excitability was an indirect measure of presynaptic depolarization of the axonal endings in oralis of the caudalis neurons.

This report deals with verification of the proposed affect code (see Fed. Proc., 37: 2246-2250) which describes how certain sensors in the thalamus may code for emotional state. In short, it predicts how reward input B interacts with the aversive unit. The affect code is one that is able to explain how reward input B interacts with the aversive unit. We chronically implanted cats were trained to self-stimulate for medial forebrain bundle stimulation (MFB) (reward input A) and to escape from midbrain reticular (RET) stimulation (0.5 sec, 50 Hz trains). During these behavioral tests, 335 units were recorded from medial thalamus and overlaying cortex, with stereotaxically guided, moveable tungsten microelectrodes. Each unit was also tested with random presentations of the MFB and RET trains and of both simultaneously so that interaction of the effects could be examined. Separate tones distinguished self-stimulation and escape periods. The animal's enclosure was sustained so that a tension transducer provided continuous data on gross movements (slight turning of the head, positioning, etc.). Unit, movement, and bar pressing data were tabulated over real time by a MDP 11 computer.

In the above model, escape was considered to be "reward input B". Since the RET stimulation was demonstrated to be aversive, and the major effect of the unit was the withdrawal of the bar press which initiated a 10 sec escape period, it was reasoned that the decreased firing may code for reward in a unit (Type I) if its activity was increased by the typical excitation after a randomly given RET train in a comparable post-stimulus period. In Type II units, post-escape activity was greater than the average unit activity following a RET train (activation of pre-escape RET trains). If inhibition codes reward in Type I units, then MFB trains would be predicted, according to the affect code, to produce an induction of pre-escape RET trains. If inhibition codes reward in Type II units, then MFB trains would not have this effect on Type II units. Indeed, they summed with the excitatory effects of pre-escape RET trains on an arousal pattern (see ref.) characterized by similar responses to the motivational opposite MFB and RET stimuli.

These results show that the affect code can be used to predict unit responses to motivational stimuli. NIH Grant RR-06102

PAIN ADAPTATION AND HYPERALGESIA DURING THERMAL STIMULATION OF HUMAN SKIN. B.H. Wootton, G.J. Robinson and J.G. Thalamann*, Dept. of Anesthesiology, Yale University, New Haven, Conn. 06510.

In these studies we measured the time course of changes in human sensitivity to pain during and following intense heating of the skin. Human subjects, each of whom gave informed consent to an approved protocol, made continuous category judgments of the magnitude of warmth and pain during constant temperature, localized heating of the skin. For heat stimuli of 38 to 47°C (10 min duration) or 50°C (1 min). Stimuli greater than 45°C were perceived as painful throughout the presentation, with magnitude ratings of warmth and pain remaining constant or increasing slightly. Pain evoked by stimuli of less than 45°C was transient and adapted (disappeared) within 1 min. In some experiments, the capacity to adapt and to rate both (C-1) and (C-1.6) superimposed on these sustained temperatures was also determined. Thresholds near the beginning and the end of each stimulation were not appreciably different regardless of base temperature.

In other experiments we obtained magnitude ratings for test stimuli (0.1-1.6°C above a base of 38°C) delivered before and at varying intervals of time following a conditioning stimulus (CS) of 50°C. Durations of the CS varied from 5 to 100s. For a CS of less than 30s, magnitude ratings of warmth and pain were typically lower (suppression) by an amount equivalent to 3-6°C immediately following the CS and recovered to normal over the next 30 min. For CS duration of 50s or more, we observed suppression for the first 90 s post CS, but following this, a return to normal occurred. Incidentally, the suppression was followed by an increase in magnitude ratings of both warmth and pain.

The development of suppression and hyperalgesia following a CS of 50°C for 60s corresponded in time and in magnitude to the sequence of fatigue and sensitization observed in G-fiber mechanoreceptor nociceptive afferents (QHs) in the monkey (Thalamann and LaMotte, this vol). The adaptation of pain during sustained thermal stimulation may be accounted for by fatigue of QHs while the failure to adapt at higher temperatures may result, in part, from a combined input from CNHs and an increasing recruitment of sensitized A-fiber nociceptive afferents. (Supported by NIH grant NS 14624)


The primate spinothalamic tract terminates in several thalamic nuclei, including the medial part of the ventral posterior lateral nucleus (VPL). To study the possibility that neurons in this nucleus are capable of transmitting nociceptive information, we have recorded from the same monkeys used for lateral posterior nucleus (VPL). The animals were anesthetized with n-chloralose and an infusion of sodium pentobarbital (4 mg/kg/hr). Recordings were made with tungsten microelectrodes, and the position of units marked with electrolytic lesions. Noxious stimuli applied to the cutaneous receptive fields included intense mechanical stimuli and graded noxious heat pulses from 35°C adapting temperature to 43°, 45°, 47° and 50°C. Noxious stimuli included hair movement, touch, rotation of joints, tapping and vibration. Many of the thalamic units were activated antidromically from the SI cortex using either a platinum ball electrode for surface stimulation or a steel microelectrode for stimulation within the cortex.

To date we have recorded from 94 thalamic neurons of these, 61 were excited by noxious heat pulses. The remaining neurons responded to innocuous stimuli, and, of the five tested, none responded to noxious heat pulses. Reconstructions from electrotic lesions indicated the cells responsive to noxious stimuli were somatotopically organized, those from the hindlimb being more laterally and those from the forelimb more medially in VPL. An ascending series of noxious heat pulses produced stimulus-response functions similar to those of spinthalamic tract neurons. In two experiments, lesions of the dorsolateral fasciculus on the side of the receptive field of some of these neurons failed to produce no observable changes in the responses of thalamic cells to noxious heat pulses, whereas in one case a lesion of the contralateral ventrolateral nuclei completely abolished these responses.

We were able to activate antidromically 25 thalamic cells that responded to noxious heat pulses by surface stimulation of the SI cortex. Of these, 11 were also antidromically activated by microstimulation within or subjacent to the SI cortex.

Based on these findings, we speculate that neurons located in the VPL nucleus of the primate receive input from the spinothalamic tract and transmit nociceptive information to the somatosensory cortex.

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The change in response to increasing drug dose may be discrete (quantal) or continuous (graded). Most researchers have assumed that opiate analgesia is a graded process. However, D'Amour and Smith (J. Pharmacoll., 1941, 2, 74) and others, using the rat, have demonstrated a quantitative suppression of tail flick by opiates. We present evidence that morphine analgesia in humans with clinical pain may also be quantal.

We administered one 2 mg/kg dose of morphine to male patients between the ages of 18 and 34 were studied. All patients underwent extraction of impacted third molars, without narcotics. Details of the procedure have been published (Levine et al., Lancet, 1978, i, 654). At two hours following surgery all patients received a placebo (an equal volume of intravenous saline) and at three hours either a placebo again or a hidden intravenous injection of 4, 6, or 8 mg of morphine. Pain was evaluated using a visual analog scale (10 cm, blank line; ibid.).

Blind administration of morphine produced a dose response curve which was monotonically over the range studied. However, individual patient's pain reports 50 minutes following morphine injection were clustered around two separate mean levels. The mean pain level of one of these clusters was above and the other below the pre-treatment mean. These clusters were defined as morphine responders and non-responders, respectively.

The mean pain levels for responders and non-responders is independent of morphine dose. We found that for increasing dose, the decrease in mean pain is due to a larger percentage of morphine responders to noxious pain (and a smaller percentage of non-responders). We have observed a similar division into two groups following placebo administration (Levine et al., PNAS in press). The mean pain levels for morphine responders and non-responders (and non-responders) are not significantly different.

We conclude that opiate analgesia is a quantal process and that it may share a common neural circuit with placebo analgesia.
EFFECT OF MORPHINE, PIMOZIDE AND NALOXONE ON RESPONDING BY RATS IN A SIGNAL DECISION PARADIGM: 23 DAY EFFECT OF PIMOZIDE. V. A. Lewis and L. Cathliner. Department of Pharmacology, Dental Branch, University of Texas, Houston, 77025.

Signal detection theory has been employed in the evaluation of behavioral response to foot shock and foot shock stimulation. The innate components from the decision making or psychological components of the response. In this study, a signal decision paradigm has been adapted to assess the effect of analgesic and antipsychotic drugs on a foot shock escape response in rats. Four groups of 10 male Sprague-Dawley rats were trained to escape a 1.2 ma. foot shock by climbing onto a platform (15 × 9 × 3 cm). After training, the effect of the narcotic-antagonistic morphine (1.3,10 µg/kg), the selective dopamine-antagonist pimozide (0.08,20,46 µg/kg), the narcotic-antagonist naloxone (0.1 µg/kg) and vehicle control were assessed on escape from tone and shock (HIT and tone and no shock (False alarm, FA) contingencies. The frequency of escape and latency to escape were recorded for HITs and FAs. During any session only one drug and one shock probability was presented and 5 day rests were scheduled between sessions to minimize residual effects produced by the drugs.

Analysis of variance revealed significant effects for morphine and pimozide but not for naloxone, vehicle control or the different probabilities of shock. Morphine at 3 and 10 mg/kg significantly reduced responding to both HITs and FAs and increased the latency for response to shock. Morphine primarily increased the withdrawal criterion. Pimozide significantly lowered HIT and FA probabilities and prolonged escape latencies compared to the vehicle control group, but not when compared to its within group vehicle control. The data suggested that pimozide had an enhanced behavioral effect lasting longer than 5 days. A pimozide time course study (0.64 µg/kg S.C. in 0.1 acetic acid) demonstrated that pimozide maximally depressed responding between 2-3 hours post injection, but did not control by day 5 but FAs were significantly depressed for 23 days and returned to control levels after 36 days. These studies demonstrate that morphine and pimozide are pharmacologically distinct mechanisms and that dopamine is important in the generation of FAs. In addition, it has been shown that pimozide may be a suitable agent for studying the long term effects of dopamine receptor blockade.

SENSATIONS AND MASSETERIC INHIBITORY PERIODS PRODUCED BY ELECTRICAL TOOTH PULP STIMULATION. Patricia A. McGrath, Tali Shemer, Richard H. Gracey and Ronald Dubner (SPON: W.H. Faller). Neurobiology & Anesthesiology Branch, NIDA, NIH, Bethesda, MD 20205.

Electrical tooth pulp stimulation in humans evokes inhibitory periods in the on-going masseteric activity, that are usually regarded as indices of pain sensation. This study investigates both the sensations produced and the masseteric inhibitory periods evoked by electrical tooth pulp stimulation in 30 subjects. Stimulation consisted of a train of 30 monophasic, monopolar, 1 msec duration, cathodal pulses delivered at 2 sec intervals. ENG masseter activity, from surface electrodes, was rectified and averaged for the 30 pulses at each stimulus intensity. Three configurations of inhibitory periods were produced: Single, (S), a single inhibitory period with a latency of 10-15 msec and duration of 10-20 msec; Double, (D), two inhibitory periods separated by a burst of muscle activity, the first having a latency and duration similar to S and the second having a latency of 40-50 msec and a duration of 10-20 msec; and Merged, (M), an elongated period in which the inhibitory periods of S seemed to merge. These inhibitory periods were correlated with stimulus strength, not magnitude of sensation; S and D periods were evoked at low and moderate stimulus intensities while M periods were evoked at high intensities. Non-pain and pain sensations were dissociated from inhibitory periods by the administration of a narcotic, fentanyl (1.08 mg/45 kg body weight), in 15 subjects. Fentanyl significantly increased the latency and magnitude of the inhibitory periods and significantly reduced the magnitude of sensations in all subjects, but there were no changes in the latency, duration or configuration of the inhibitory periods. Non-pain and pain sensations were also dissociated from inhibitory periods by the administration of a narcotic, fentanyl (1.08 mg/45 kg body weight), in 15 subjects. Fentanyl significantly increased the latency and magnitude of the inhibitory periods and significantly reduced the magnitude of sensations in all subjects, but there were no changes in the latency, duration or configuration of the inhibitory periods. Non-pain and pain sensations were also dissociated from inhibitory periods by the application of an electrical conditioning stimulus (CS) to the central incisor in 30 subjects. A 30 pulse CS train (300 µA ± 25), produced a significant elevation in non-pain and pain thresholds and a significant reduction in magnitude of sensation in the subjects tested. The 30 pulse CS train was also effective in changing the masseteric inhibitory periods. The administration of an opiate antagonist, naloxone, 10 mg, partially reversed the suppression of sensation by the CS, but the data suggest that the masseteric inhibitory periods may be independent of any detectable tooth pulp sensation; 2) narcotic manipulations reduce tooth pulp pain sensitivity but inhibit the inhibitory periods; 3) the inhibitory periods involves trigeminal brain stem pathways distinct from those that mediate pain sensations; and 4) the suppression of tooth pulp non-pain and pain sensations by conditioning stimulation may be mediated by endogenous opioid pathways.

STIMULUS-DEPENDENT CHANGES IN FOOTSHOCK SENSITIVITY FOLLOWING MEDIAL FOREBRAIN BUNDLE LESIONS IN THE RAT. Carlton E. Links, Leonard M. Menéndez, Dept. of Psychol., Northern Illinois Univ., DeKalb, IL 60115.

Forebrain serotonin (5-HT) depletion has been reported to produce decreased footshock sensitivity to footshock in the rat, whereas forebrain serotonin (5-HT) depletion produces hyperalgesia as measured by the hot-plate technique. Thus, there appears to be some degree of specificity for lesioning of the medial forebrain bundle which results in reduced footshock sensitivity and lesioning of the prefrontal cortex which results in increased footshock sensitivity. In this study, medial forebrain bundle (MFB) lesions disrupt the forebrain levels of both of these amines and have been reported to produce both types of hyperalgesia. However, difficulty in replicating the effects of MFB lesions on footshock sensitivity in the laboratory suggested that there might be considerable stimulus sensitivity within the forebrain MFB system mediating this effect. To test this possibility normal (S), sham-operated (SHM) and MFB-lesioned rats (MFB) were tested for sensitivity to footshock using the flinch-jump technique and two different constant current shockers that differed in terms of the nature of the current delivered to the grid floor of the test chambers (ac or dc). The animals were then retested with the ac shocker using three different shock durations (.05, 1 and .2 sec) with each of two different shock series (REG & Half). The REG series was the same series of shocks used in the initial tests and the Half series used current intensity increments of 20% smaller than those of the REG series. The animals were always retested in the same chamber. Following behavioral testing the forebrains were assayed for 5-HT and NE levels and the brainstems were removed for histological verification of the locus and extent of the lesions.

The lesions were well localized and produced 47% and 40% depletions of forebrain 5-HT and NE respectively. The MAO levels of the MFB group were significantly lower than those of the SHM group with both shockers, and there were no significant effects for the REG & Half series. The shock thresholds of the MFB group were also significantly lower than those of the N group, but this was not the case for the shock latency, with the ac shocker. The shock threshold of all three groups decreased significantly as shock duration increased. However, only the .1 sec shocks in the REG series produced a significant lowering of the MFB jump thresholds. The ac shocker thresholds of the MFB rats were never significantly lower than those of the control groups with the Half series. Flinch thresholds of the sham-operated rats were significantly lowered by the .1 sec shocks in the REG series. No significant changes were observed in the latency, duration or configuration of the inhibitory periods. These data suggested that pimozide had a pharmacologically distinct mechanisms and that dopamine is important in the generation of FAs. In addition, it has been shown that pimozide may be a suitable agent for studying the long term effects of dopamine receptor blockade.

Stimulation of the mesencephalic periaqueductal grey (PAG) results in an enhancement of pain threshold which is dependent upon the integrity of serotonin-containing neurones. In view of the fact that PAG stimulation causes inhibition of spinal interneurons, it is possible that stimulation in the PAG causes a reduction in the production of descending inhibitory systems. The axons of serotonin-containing neurones of the raphe magnus (RM), unlike those of the mesencephalic dorsal raphe, project to the spinal cord. Stimulation of the RM has been shown to cause analgesia in the rat and cat. The RM might therefore be an essential region mediating the inhibitory influence of PAG stimulation upon spinal neurones.

Experiments were carried out to determine the effect of RM stimulation upon intrinsic spinal neurones in urethane-anaesthetized rats. Two populations of cells were studied: (a) spinobulbular tract neurones, identified by antidromic activation by spinal application of the convulsive stimulus thalamic (c) unidentified spinal neurones. Most of these cells responded to cutaneous stimulation. They could be activated by both weak, tactile stimuli, and by intense, noxious inputs. The response pattern to electrical cutaneous stimulation consists of a short latency (5-10 msec) burst of action potentials to weak stimuli and a late (>50 msec) discharge to high stimulus intensities. Trains of stimuli applied to RM (20-200µA; 20Hz; 0.2 msec; train duration 45 msec, starting 50 msec prior to the cutaneous stimulus) inhibited the responses to weak cutaneous stimuli in 65% of cells tested and those to intense stimuli in 67% of neurones. The mean, threshold, RM stimulus intensities required to inhibit responses in 'non-responders' were lower than those required in RM neurones, 11.0µA and 15.3±16.0µA respectively. The difference between these values is significant (P<0.02; t-test). In contrast to the findings obtained with monkey and cat, RM stimulation was more effective in inhibiting responses to weak cutaneous stimuli than those to intense inputs. RM stimulation was shown to be equipotent with the antidiromically evoked action potential in 53% of the spinobulbar tract neurones. This implies that the inhibitory effect of RM upon responses to cutaneous inputs may be a consequence of a direct post-synaptic influence on these cells. (Supported by the Medical Research Council of Canada.)


The phenomenon of stimulation produced analgesia (PSA) has been characterised by inter-subject variability both in post-stimulation duration of SPA and in naloxone's ability to block it. To examine the interaction of PAG and RM, post-stimulus time histograms were recorded of RM unit activity while electrically stimulating the PAG (PAG) and the median raphe nucleus (RM) and were tested again at least 1 week after surgery. Most animals later received lesions of the n. raphe magna (NRM) and were tested again at least 1 week after surgery. Spinal responses in these experiments, RM neurones were tested for spinal projection by electrically stimulating the lumbar DLF. RM neurones were designated as raphe-spinal if DLF stimulation produced antidromic responses which were abolished by naloxone. A total of 67 neurones were studied in all experiments. The distribution of responses was found to be: 54% facilitation, 36% depression, 12% facilitation and inhibition, and 9% no response. The mean latency to onset of facilitated response was 12.0±1.6 ms (mean±S.E.M.) and the mean latency to onset of inhibition was 12.3±1.7 ms. RM neurones tested, 26% were shown to have spinal projections (mean conduction velocity was calculated to be 14.6±4.6 mm/s). No other differences were seen in comparing RM neurones with those of the PAG in terms of latency. PAG neurones were shown to have a facilitatory response to PAG stimulation and no significant difference was seen in comparing RM and PAG neurones. For seven cells, the extent of current spread at the stimulating electrode was measured. The current that activated the PAG electrode 1,2, or 3 mm dorsally or ventrally and recording additional post-stimulus time histograms. In all cases, moving the stimulating electrode 3 mm ventrally reference but no response at 3 mm ventral to reference. On the whole, these data show electrical stimulation of PAG often leads to synaptic activation of RM neurones. This lends further support to the hypothesis that activation of raphe-spinal neurones mediates spinal anti-nociceptive responses to PAG stimulation.
Electrolytic lesions of the nucleus raphe magnus (NRM) result in hyperalgesia and attenuation of morphine-induced analgesia (MIA) when tested between ten days and two weeks following surgery (Proudfit and Anderson, 1975). In subsequent studies, several animals of each group were killed before seven days following the lesion to determine if any of the lesions, showed no alteration in MIA. Therefore, we examined the time-course of lesion effects on both nociceptive threshold and MIA. Following successful, either the NRM or raphe pallidus, rats were tested at seven day intervals for 35 days using the tail flick test. Unoperated control rats were similarly tested at seven days after injection. The nociceptive threshold was reduced by 33% and by 14 days by 42% of control. However, testing at subsequent seven day intervals showed a gradual recovery and return to control by day 35.

The effect of lesions on MIA (5 mg/kg) was quite different. No effect was seen at seven days, but testing at subsequent seven day intervals revealed a gradually increasing attenuation of MIA throughout the 35 days of testing.

The effect of reversible lesions of the NRM produced by the microinjection of the local anesthetic tetracaine (TET; 5 µg in 0.5 µl of saline) was also examined. Rats were implanted with chronic indwelling microinjection guide tubes (25 ga) and seven days after surgery the nociceptive threshold was determined and morphine sulfate (5 mg/kg, sc) was injected. At the peak of MIA (30 min) TET was slowly infused through a 28 ga injection cannula into the NRM. Tail flick latencies determined 4, 15, 25 and 35 minutes after injection were not significantly different from those determined before TET. However, when TET was injected locally in the absence of morphine pre-treatment, the animal's reaction time was at least doubled. This effect was evident immediately after the injection, but declined to near normal reactivity by 45 minutes.

The results suggest that raphe lesion-induced attenuation of MIA and hyperalgesia are mediated by the destruction of separate neuronal systems. In addition, the failure of acute raphe lesions or TET to produce a significant reduction of the nociceptive threshold indicates that the NRM to affect MIA indicates that the NRM is not involved in mediating the antinociceptive actions of opiates. It is likely that an effect secondary to destruction of the NRM is responsible for its attenuation of MIA observed following chronic NRM lesions.

**Comparison of Alternating versus Direct Current Electroanalgesia**

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We have shown that trains of rectangular pulses, of 10% duty cycle at 1000Hz, capacitively coupled to effect alternating polarity, can block affective aspects of cat tooth pain when applied to exposed dental pulp during 50-70 seconds. Direct current of similar strength (Fields et al., Abstr. Soc. Neurosci, 4:459, 1978). For clinical use for electroanalgesia (EA), a low duty cycle (10%) are conceptually superior to direct current (Fields et al., Oral Surg. 38:694-703, 1972) because of lower power dissipation (small duty cycle) and reduced monodirectional iontophoresis (alternating polarity). In unpublished observations, we have also found that neither alternating nor direct currents ranging to 1000µA cause histologically detectable pulp aberrations when applied to intact teeth (through the enamel). Such current application simulates a practical clinical EA protocol, but it is not known if it truly induces affective unit hyperexcitability.

This question was addressed in the present studies in which excitability of single pulp-driven units in cat Gasserian ganglion was observed during and following application of 0-500µA dc (1000Hz, 10% duty cycle rectangular pulses, capacitively coupled) and dc EA current to intact maxillary canine teeth. The excitability index was determined by electrical stimulation via a pair of dental electrodes (e.g., Fields et al., Exp. Neurol. 53:386-398, 1976). For both ac and dc waveforms a progressive rise in pulp-driven unit activity, compared to pre-EA control, was seen with increasing current over the 0-100µA range. Threshold elevation of at least 500% was seen for both waveforms at 100µA after as little as 1 minute duration. Recovery to control threshold following 1 minute of dc at 100µA required as much as 10 minutes; for the majority of units examined, recovery periods following ac were shorter than following dc of similar intensity.

These results demonstrate that a) both ac and dc EA currents applied to intact enamel of cat maxillary canine teeth are capable of attenuating activity of pulp-driven units in the Gasserian ganglion, and b) the ac EA employed exhibited adequate efficacy but superior recovery characteristics when compared to dc of similar intensity.

**Intracerebral Substance P in Mice: Behavioral Effects and Narcotic Agents**

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In preliminary studies with mice, acute intracerebral injection of substance P was found to induce a unique behavioral phenomenon characterized by reciprocal hindlimb scratching movements. In mice weighing 18 to 22g, an injection site approximately midline at the level of the external auditory meatus, using a 27 gauge hypodermic needle, was able to cause a dramatic and consistent responses. Erythrocyte markers indicated injection directly into the IIIrd ventricle with the solution (2 µl) spreading to all cerebral ventricles.

Acute intracerebral injections of substance P induced distinct behavioral changes whose intensity appeared dose-related. Within 60 to 90 seconds, the mice seemed agitated, pressed against the cage walls, and engaged in reciprocal hindlimb scratching movements directed toward the sides of the upper body and jaw areas. These latter responses were pathognomonic, being interspersed with periods of exaggerated preening and biting at the abdomen and hindquarters. The substance P-induced reciprocal hindlimb scratching response was the most consistent and reproducible of the behavioral responses observed, generally occurring within 2 minutes and always within 5 minutes after intracerebral injection. Injury, shock, or manipulation of the animal elicited a scratching episode observed in 5 minutes after intracerebral injection as a positive response, the ED50 was for substance P = 0.06 µg/mouse. Similar dose-response curves were obtained for the related undecapeptides physalaemin (ED50 = 0.025 µg/mouse) and leedolisin (0.004 µg/mouse), but not by several unrelated peptides (TRH, neurotensin, somatostatin). Analgesic narcotic agents with predominant agonist activity administered intraperitoneally prevented the reciprocal hindlimb scratching response, whereas substance P (0.25 µg/mouse) was similar to that reported for binding to rat opiate receptors in vitro. Narcotic agents with predominant antagonist activity (naloxone) were effective while the narcotic antagonist naloxone completely reversed the action of morphine.

Evidence suggests that the undecapeptide substance P may serve as an important transmitter or modulator of sensory processes, and its action may be associated with algesia or analgesia.

**Profound Potentiation of Morphine's Analgesic Potency by Concurrent Intrathecal and Intraventricular Administration**

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To examine the mode of interaction between the spinal and supraspinal narcotic-sensitive structures in the mediation of analgesia, we have measured the antinociceptive effect [hotplate (HP) and tailflip (TF) methods] produced by intrathecal (i.t.) and intraventricular (i.vt.) injections of morphine sulfate. Rats in which the fourth ventricular exits had been acutely occluded received an i.t. (lumbar) or i.vt. (lumbar spinal subarachnoid space; 0-10 µg in 5 µl) injection of morphine which could conceivably be obtained when morphine is given systemically administered morphine and that neither action locus can be considered the "primary" site of action.

The effect of thermal, mechanical and other forms of tooth stimulation encountered by normal mammals have been extensively studied and shown to involve characteristic patterns which can be recorded by electrodes in denta1 cavities. Whereas the response to cold stimulation was similar to that observed when cooling an anoc, the effect of heat showed marked differences which could be attributed to the special ized receptor. Davies (1969) examined the excitability of denta1 receptors in the cat tooth in response to heat and found an increase of excitability when extracellular potassium was decreased and an inhibition when it was increased. These observa tions have been confirmed and extended. However, increases in extracellular sodium concentration have only resulted in comparable increases in excitability. Since the sensory re sponse to heat is only slightly affected by topical application of tetrodotoxin (TTX), sodium may not be a major contributor to the transducer process. This finds support in the report by Hockett (19) that intracellular potassium is the odontoblast process may be significantly above the level in dentinal fluid. A scheme for transducer function consistent with these obser vations is proposed.

2097 LOCAL BRAIN GLUCOSE UTILIZATION EVOKED BY DENTAL PULP STIMULATION IN CATS: A COMPARATIVE STUDY. Andrew G. Shetter and Carol Kreinick.* Neurosurgical Research Laboratory, Barrow Neurological Institute, Phoenix, AZ 85013

The pattern of local brain glucose uptake produced by dental pulp stimulation in cats and rats was investigated using the 2-deoxyglucose method of Sokoloff. Bipolar electrodes were inserted into the pulp cavity of either a mandibular or maxillary incisor tooth under pentobarbital anesthesia. Constant current 100µA electrical pulses were delivered at an intensity sufficient to produce a visible jaw jerk reflex. An intravenous injection of 2-deoxyglucose (10 µCi/100µg) was given and continuous stimulation was performed for 45 minutes. Autoradiographs were prepared from unfixed sections of the brainstem and upper cervical spinal cord, and the resultant optical density patterns were analyzed with the aid of a micro densitometer. Control groups included animals who had electrodes implanted but not stimulated, and those in whom maxil lar stimulation was performed after surgical ex nuclation of the pulp cavity. Stimulus-related increases in glucose uptake were seen throughout the entire trigeminal sensory system in both species, extending from nucleus caudalis at C1-2 to nucleus oralis - minus sensory nucleus in the rostral midbrain. Enhanced glucose metabolism was less pronounced in nucleus interpolaris, and no changes were apparent in the trigeminal mesencephalic nucleus or the motor nucleus. The increases in metabolic activity produced by dental pulp activation in the rat were more ventrally and covered a larger area. This somatopic distribution was apparent in both species, but was more discrete in the rat than in the cat. No alterations in glucose uptake were detected in extra-lesional or reticular pathways for any of the animals studied. The 2-deoxyglucose technique seems to be a useful means of investigating trigeminal nociceptive systems.


We have recently found that electrical stimulation of the periaqueductal gray (PAG) or nucleus raphe magnus (NRM) can cause a powerful suppression of the digastric (jaw-opening) reflex and primary afferent depolarization (PAD) of tooth pulp afferent endings in trigeminal (V) brainstem nuclei oralis and caudalis. Many recent studies have implicated nucleus caudalis in the relay of orofacial pain, and PAG and NRM in endogenous opiate-related mechanisms of analgesia. Moreover, nucleus caudalis receives a direct projection from PAG and NRM, and indicates that PAG and NRM may be involved in functions other than analgesia. (Supported by NIAAA).


Much emphasis has recently been placed on the role of the periaqueductal gray matter (PAG) and nucleus raphe magnus (NRM) in endogenous opiate-related mechanisms of analgesia. Little consideration has so far been given to possible interactions between these two sites on functions other than those involved in pain and its control. Yet projections from one or both these sites, as well as opiate receptors, have been found in regions concerned with functions other than nociception, e.g. the solitary tract nucleus (STN), the site of respiratory reflex interneurons and some respiratory control centers. Thus it might be expected that the reflex inhibition and PAD effects are specifically induced by the activity of such STN neurons, and respiration and related reflex functions, are influenced by PAG and NRM stimulation and if so observed could be reversed by the administration of the opiate antagonist naloxone.

In anesthetised cats, we first determined if some of the functions that are sensitive to inhibition, such as coughing, could be influenced by PAG and NRM stimulation. Only a transient depression of respiration was observed with PAG and NRM stimulati, but marked decrease occurred in the incidence of coughing and swallowing elicited by stimulation of the superior laryngeal nerve or upper respiratory tract; naloxone (0.4 mg/kg, i. v.) could reverse these PAG and NRM induced depressi ves effects. The effects were reflected in changes in the activity of functionally identified single neurons recorded during microelectrode penetrations of STN. These neurons were characterized by their respiratory-related rhythmic activity, or their short-latency reflex responsiveness to low-threshold superior laryngeal or vagal nerve stimuli. The rhythmic activity of respiratory neurons was depressed during PAG and NRM stimulation, although a decrease in the peak firing frequency of each rhythmic burst was regularly observed. In contrast, the reflex responses of STN neurons could be powerfully suppressed by PAG and NRM stimulation, and the suppression could be reversed by naloxone. These studies indicate that respiration and in particular the associated reflex activities of coughing and swallowing are depressed by endogenous opiate-related influences derived from PAG and NRM. Such effects may be associated with the depression of respiration and coughing that can result from opiate overdose. The depressive effects appear to be reflections of the suscepti bility of STN neurons that are involved in respiration and associated reflex activities to inhibitory influences from PAG and NRM, and indicate that PAG and NRM may be involved in functions other than analgesia. (Supported by NIAAA).
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and 17 AMHs and 9 warm (C) fibers innervating the hand or foot. Of discharge that increased gradually. The warm fibers responded those of CMHs averaged 45º. A conditioning stimulus (CS) of 50º following intense local heating of the skin. Twenty-three only during the first few seconds of the stimulus. After termination of the CS, C-fibers (CMHs) and 25 A-fibers (AMHs) were classified as sensitive to noxious mechanical and noxious heat stimuli.

C-fibers (CMHs) and 25 A-fibers (AMHs) were classified as sensitive to noxious mechanical and noxious heat stimuli. Considerable evidence has been presented to impl­lying the activation of descending pathways from the brainstem to the spinal cord. A conditioning stimulus (CS) of 50º (60s duration) was delivered to the receptive fields of 14 CMHs sensitive to noxious mechanical and noxious heat stimuli. Twenty-three animals received a baseline bar test followed by a flinch-jump test followed by a second bar test (taking 10 min). They were then injected with drug (either water or etorphine in a 1 ml so­lution) into one neuroanatomical location. All animals received both water and etorphine, presented in a counterbalanced order and separated by a four-day interval.

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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 microinjec­tions were made into one of a number of sites. Microinjections of etorphine into the periaqueductal gray, midbrain reticular formation, cerebral aqueduct; cerebellum; cau­date putamen; basolateral amygdala; cortico-medial amygdala, glo­balus lateralis, medial thalamus, hippocampus. Each site was re­presented by a minimum of 6 subjects.

The flinch-jump technique was used to assess pain sensitivity and the bar test was used to study catalepsy. Etorphine was ad­ministered in a 1 ug dose. Each animal was used only once in one experiment and was administered etorphine only one time. The animals received a baseline bar test followed by a flinch-jump test followed by a second bar test (taking 10 min). They were then injected with drug (either water or etorphine in a 1 ml so­lution) into one neuroanatomical location. All animals received both water and etorphine, presented in a counterbalanced order and separated by a four-day interval.

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ECTOPIC GENERATION OF IMPULSES IN PERIPHERAL SENSORY NERVE FIBRES IN MAN. Erik Toröebrink and José Ochoa. University Hospital, Uppsala, Sweden, and Dartmouth Medical School, Hanover, NH, USA.

Although much is known about pathological peripheral nerve fibres as abnormal conductors of impulses, little is known about them as abnormal generators. There are some insights into abnor­mal spontaneous activity in motor nerve fibres, but knowledge about similar activity in sensory nerve fibres in man is virtually nonexistent. This paper reports abnormal activity in single peripheral sensory nerve fibres and it is suggested that the abnormal, spontaneous activity reflects a failure of the normal sensation, in volunteers experiencing a variety of pares­thesiae.

Method: Paresthesiae were induced after release of a phymo­someter cuff inflated above systolic blood pressure round the arm for about 30 minutes. Single unit impulses were recorded from sensory nerve fibres and differences between records made during this and other studies will be discussed. Results suggest a pos­sibility for separate neurological substrates for analgesia and catalepsy. Additionally, results show at least some site-specificity for analgesia and catatonia after etorphine injection, suggesting that the effect is not just a result of gross diffu­sion following microinjection of a lipopholic substance in the brain.
2103 INTRACARDIAC BRADYKININ INJECTIONS AND THEIR EFFECT ON SPINAL NEURONS HAVING VISCEROSOMATIC CONVERGENCE. R. Neal Weber* and Robert D. Foreman, Dept. Physiology & Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190.

Previous studies from this laboratory have shown that the neural discharge rate of spinal neurons either increased or decreased during occlusion of the left coronary artery or the pulmonary artery. The purpose of this study was to determine whether an algic substance such as bradykinin would influence the discharge rate of these cells when the substance was injected into the heart. The activity of single cells was recorded extra-cellularly from the left gray matter of the T2 and T3 spinal cord segments in 12 chloralose anesthetized cats. All the cells responded both to mechanical manipulation of the somatic receptive field and to electrical stimulation of the left T2 to T3 sympathetic chain. These cells responded to noxious pinch when it was applied to the receptive field of the left forelimb and chest. After the cells were tested for viscero-somatic convergence, bradykinin was injected into the chamber of either the left atrial appendage or left ventricle. The injected concentration of bradykinin varied between 100 µg/ml and 1 mg/ml in doses of 0.2 to 0.3 ml giving a total of 20 to 300 µg per injection. A total of 31 cells responded to both somatic manipulation and sympathetic stimulation. Fourteen of the 31 cells responded to the chemical injection while the remainder showed no change in their discharge pattern. Of the 14 cells, 13 were excited and one was inhibited following transection of the left sympathetic chain. These cells responded to noxious heat when it was applied to the receptive fields and from visceral receptors activated by algesic chemical stimuli. The results lead to the suggestion that injections of bradykinin caused the excitation of cardiac receptors whose afferents are associated with the descending serotonergic system and a second portion is located in primary afferent terminals, the latter of which is depleted intrathecally. With regard to nociceptive transmission this data suggests that there are two populations of sP containing spinal terminals, one is associated with the descending serotonergic system and a second portion is located in primary afferent terminals, the latter of which is depleted intrathecally. With regard to nociceptive transmission this data suggests that sP may be associated with primary afferents which carry information related to heat and peripherally applied algic chemicals. This work supported by NINDS 14629.


Following the rationale that sP may be a nociceptive transmitter in primary afferents, its depletion in the spinal cord might result in analgesia. C given systemically can deplete sP in the cord. We therefore sought to examine the effect of this drug administered intrathecally on the nociceptive threshold and sP levels in the rat. To carry out these experiments, rats were chronically implanted with intrathecal catheters inserted through the cisterna magna into the lumbar subarachnoid space. Following a 7 day recovery, animals received C (3 or 30 µg/15 µl of 50% salmine-dimethylsulfoxide vehicle) or vehicle. The injection of C, but not vehicle produced a 2-phased dose-dependent response: the first, lasting 1-3 min was characterized by muscular contractions; in the second phase the animal regained voluntary control and exhibited intense gnawing and scratching of the caudal regions of the body (15-30 min). Following these phases the animals displayed no unusual motor signs and exhibited normal reflex function. Subsequent nociceptive testing revealed that 60-70% of the animals receiving 30 µg C showed no response to otherwise noxious thermal stimuli as measured with the 55°C hot plate or tail flick. These animals showing significant blockade continued to show no response for periods up to 3 months after C treatment (until natural death or sacrifice). The aversive behavior produced by subcutaneous injections of formalin (0.1 ml/25) was substantially reduced but not blocked in C-treated animals as compared to non-treated controls. In contrast to the elevated thermal and chemical thresholds, these C-treated rats displayed a normal response to mechanical pinch applied to the paws. Intrathecal C produced a dose-dependent depression to 50% in the spinal but not hind brain levels of sP. Comparison of degree of thermal analgesia with degree of depletion of sP indicated a highly significant correlation. I.V. administration of 30 µg of C produced no change in either sP levels or in the nociceptive threshold. Intrathecal 5,6-dihydroxytryptamine (20 µg in 15 µl ascobic acid vehicle) produces a 70-90% decline in spinal 5-HT and a 40% decline in spinal sP. This neurotoxin in contrast to C, produced a significant reduction in the nociceptive threshold. These results suggest that there are two populations of sP containing spinal terminals, a portion is associated with the descending serotonergic system and a second portion is located in primary afferent terminals, the latter of which is depleted intrathecally. With regard to nociceptive transmission this data suggests that sP may be associated with primary afferents which carry information related to heat and peripherally applied algic chemicals. This work supported by NINDS 14629.

2105 TAIL-FLICK NEURONS OF THE NUCLEUS RAPHE MAGNUS. M.D. Zaretsky, J.D. Levine* and H.L. Fields. Depts. of Neurology and Physiology, Univ. of California, San Francisco, CA 94143.

We have recorded from individual neurons of the nucleus raphe magnus (NRM) in rats performing tail-flicks in response to noxious heating of the tail. The tail-flick test has been used to demonstrate both stimulus-produced (Mayer, D.J. et al, SCIENCE 174: 1351, 1971) and outpass (Akih, R. et al, SCIENCE 191:961, 1976) analgesia. Histological studies and electrical stimulation of the NRM suggest that the NRM is a significant region of a descending analgesia pathway (Abbasam, A.J. et al, PNAS 73:6685, 1977). We now report that the NRM contains two classes of neurons with discharge patterns that are strongly correlated with tail-flicks in response to noxious heating. In the first class of neurons, tail-flick triggers, strong excitation occurs when noxious heating results in a tail-flick. Excitation of these units does not occur when noxious heating fails to produce a tail-flick. Increased firing of the nerve cells begins just prior to the onset of the tail-flick. Some neurons of this class discharge briefly, others continue to discharge after the tail-flick occurs. The second class of NRM neurons, off-units, firing spontaneously at a moderated rate, are inhibited from firing by noxious heating of the tail.
PLASTICITY

Previous studies have demonstrated that the presence of extraacellular Ca during tetanic stimulation of hippocampal afferents is necessary for the development of long term potentiation (LTP) of synaptically evoked responses in this region. Inasmuch as Ca plays a significant role in transmitter release, and an augmented transmitter output may underlie the phenomenon of LTP, the present experiments were undertaken to determine whether there is a significant uptake and retention of Ca following brief tetanic stimulation of the Schaeffer-collateral input to Ca1 pyramidal neurones which induces LTP. Transverse slices of the rat hippocampus were prepared for electrophysiological recording and incubated in a modified Ringer's solution containing 45Ca for 2 hrs prior to stimulation. Paired control and tetanized (50-100 Hz, 2-5 sec.) slices were removed at 5 and 30 min. following stimulation and washed in Tris-buffer (pH 7.4) containing 10 mM LaCl3 in order to remove surface bound 45Ca and to prevent release of intracellular Ca. Slices were freeze dried and the soluble protein dissolved in buffer and aliquots taken for total protein determination and 45Ca content. In all pairs and at both time intervals, tetanic stimulation resulted in an increased 45Ca content compared to controls. No loss of 45Ca was observed 30 min. after tetanization compared to 5 min. post-tetany. The large influx of Ca which occurs as a result of tetanic stimulation and the right that this Ca is retained at 30 min., suggests that an augmented transmitter release, dependent in part upon uptake and retention of Intracellular Ca, may underly the phenomenon of LTP.

(Supported by NRC of Canada.)


The activity originating from the annula of a horizontal semicircular canal (HSC) is increased by ipsilateral angular acceleration of the head and decreased by contralateral angular acceleration. This activity directly excites the ipsilateral medial vestibular nucleus (MVN) and indirectly inhibits the contralateral MVN via a GABA-ergic commissural pathway. This reciprocal organization can be altered by plugging one HSC. The postplug activity of the MVN contralateral to the plugged HSC would be modulated only by an excitatory signal originating from the intact HSC. The activity of the MVN ipsilateral to the plug would be modulated only by an inhibitory signal relayed through the commissural pathway. We have studied the effects of unilateral plugs of the HSC on the horizontal vestibulococular reflex (HVOR) of the rabbit, and examined the influence of intravenously administered diazepam (0.4mg/kg), a GABA agonist, on the HVOR following unilateral plugs of the HSC. An HSC was plugged by inserting a small silver spindle into the bony canal. The HVOR was evoked by sinusoidal oscillation of rabbits on a rate table (100-200 Hz 30 degrees/sec) measured with an infra-red technique. Immediately following a unilateral HSC plug, the gain (eye velocity/head velocity) of the HVOR is reduced. If "postplug" vestibular stimulation is maintained, an asymmetry in the HVOR slowly develops (3-15hr). This asymmetry reflects a relative increase in the gain of the HVOR when the eyes move toward the side of the plugged canal. Maximum asymmetry occurs in 24-48hr. The onset of the asymmetry can be delayed by postponing the initiation of postoperative vestibular stimulation. Although the average gain never recovers, the relative asymmetry of the HVOR is gradually compensated in a frequency-dependent manner. After 5-10 days this compensation is even reversed, causing a relatively higher gain of the HVOR when the eyes move toward the intact side. Intravenous injections of diazepam in normal rabbits reduce the gain of the HVOR. Diazepam injections after unilateral HSC plugs greatly increase the asymmetry of the HVOR. The time course of this diazepam-induced effect corresponds to that of the development and compensation of the asymmetry. The efficacy of the diazepam might be attributed to the relatively increased postsynaptic sensitivity of the MVN on the plugged side to GABA-ergic agents. This increased sensitivity may be stimulated by the near-exclusive modulation of the MVN on the plugged side by the GABAergic agents. (Supported by PHS Grant EY-00848 and The Oregon Lions Sight & Hearing Foundation.)


We produced a surgically induced strabismus in 11 kittens by cutting the right medial or lateral rectus muscle at different postnatal ages. Following at least one year of visual experience, we recorded from single neurons from area 17, and for each unit we determined the eccentricity of its receptive field, receptive field type (simple or complex), receptive field size and position in the visual field.

We found a gradual increase in the percentage of binocularly driven units with age at surgery. In kittens made strabismic between postnatal days 10 and 18, only 10% of the units could be driven by both eyes. Surgery performed on days 26, 36-38, and 60 gave percentages of binocular cells of 25%, 30% and 50% respectively. Following surgery on day 80, the percentage of binocularly activated units was not significantly different from that found in normal adult cats. We found no abrupt change which might signify the end of the critical period.

In kittens operated before day 42, a high proportion of those cells which were binocularly driven had complex receptive fields, which is in agreement with observations that complex cells are more resistant than simple cells to other manipulations which reduce binocularity (Payne et al. 1979).

We found some cells with abnormal receptive field properties in these kittens. In the earlier operators, we commonly found visually unresponsive cells at the borders between the eye dominance columns. In the later operators, we found binocularly driven cells with abnormally large receptive fields (up to 11x150).

The critical period for changes in binocularity resulting from surgically induced strabismus differs somewhat for the critical period for effects of monocular suture. Following monocular suture, all units will be driven only by the experienced eye even if the stimulus is unequal. As late as 6 days postoperatively (Hubel and Wiesel, 1970) whereas kittens made strabismic on day 26 retain 25% binocularly driven units. Our findings suggest: (i) some complex cells can retain their input from both eyes despite a severe eye misalignment; (ii) abnormal receptive field properties can be produced in kittens without deprivation of visual experience of form or movement; (iii) the critical period for squint ends earlier and more gradually than the critical period for monocular deprivation.

Supported by EY02488, EY0288, BNS 7724923, MH31268.


Somatic bouton rejection and dendritic branch loss can be induced byotomy. The following experiment studies the dendritic profile of rat motoneurons in the segment under the T1-T2 brance after ventral root section (Normal, 14, 30, 60, 90 days postoperative). Six animals per group were utilized and the segment of spinal cord of the crushed root impregnated by the tangle-m Silver and Golgi technique. Neurons were selectively injected with isotope to determine the size and shape of dendrites. Six independent samples were generated per postoperative day. Dendrites were analyzed for total number of branches, branches that were terminal and serpentine length (total length of entire dendrite). (Supported by NIH grants: Neuman-Keuls a posteriori) There was a significant, cyclic, loss and gain of numbers of dendritic branches (Low, 14 and 60 days, high 30 and 90 days) and numbers of branches that were terminal (Low, 14 and 60 days, high 30 and 90 days). The serpentine length of the dendrites showed the same significant cycle. These data show that following axotomy motoneurons show cyclic growth and branching.

Supported by NS 06164, NIHCS.
SPROUTING OF PERIPHERAL SYMPATHETIC NEURONS IN THE ABSENCE OF AFFERENT NEURONAL INPUT: Leslie Brother, Keith A. Crocker and James H. Davis. (Spon.: T. W. Blassie, VA Medical Center and Duke University Medical Center, Durham, N. C.)

Although it is well established that neuronal rearrangements occur after brain injury, relatively little is known of the factors that regulate such plasticity. One particular rearrangement, the appearance of peripheral noradrenergic neurons in the dentate gyrus several weeks after septal lesions, provides a useful model for the study of the regulation of reactive synaptic tosors or sprouting.

We took advantage of the accessibility of the superior cervical ganglion to surgical manipulation and cut the preganglionic input to the ganglion (decentralization) in some animals, removed the ganglion in others and carried out a sham operation on the remainder. The decentralized and sham-operated controls had a bilateral medial septal lesion placed the day following neck surgery and were sacrificed four weeks later. The ganglionectomized animals were also sacrificed four weeks after septal lesioning, but their superior cervical ganglia were removed 3 weeks after lesioning (one week before sacrifice). All decentralized animals developed an ipsilateral Horner's syndrome which could be reversed by parahyphosphatemia eye drops. Furthermore, peripheral noradrenergic fibers in the pia and on the blood vessels appeared unaffected by the decentralization. The septal region was sectioned and examined histologically to verify lesion placements in each animal. The hippocampal formations were studied with a glyoxylic acid method for visualizing catecholamine fibers.

All sham-operated controls demonstrated obvious bilateral noradrenergic sprouting identical to that in the decentralized animals. Ganglionectomized animals had no peripheral noradrenergic fibers ipsilateral to the removed ganglion, but sprouting in the contralateral hippocampal formation appeared similar to controls. Decentralization did not prevent sprouting, since all but one decentralized animal had peripheral noradrenergic fibers in the hippocampal formation ipsilateral to the decentralization.

Our data show that afferent input and neuronal activity is not necessary for peripheral noradrenergic neurons to sprout. These data further show that some factor in the target tissue initiates the sprouting response. The attenuation of sprouting in the decentralized animals is likely dependent on an interocular transfer. Since the developing animal, nerve impulse flow plays an important role in the extension or maintenance of sprouted fibers.

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INFLUENCE OF EARLY MONOCULAR DEPRIVATION ON [14C]-2-DEOXYGLUCOSE LABELLING PATTERN IN PIGEON VISUAL STRUCTURES. Andreas Burkhalter* and Peter Streit* (Spon: Michel Cuñod). Brain Research Institute, CH-8029 Zurich.

The visual behavior of pigeons, monocularly deprived for the first 8-11 months, was found to be deficient in respect to pattern discrimination and interocular transfer. Since [14C]-2-deoxyglucose probes have been shown to visualize functional activity of cerebral structures, this method was used for the study of patterns of glucose consumption after long-term deprivation in the pigeon.

Six pigeons were raised for 244 to 341 days with one eye occluded by an opaque plastic cap from the day of hatching. At the end of the deprivation period, [14C]-2-deoxyglucose were injected i.v. in 3 groups of 2 awake animals: During 45 min, both eyes were free (DBE) or one eye was covered, either the deprived one (DDE) or the nondeprived one (DDE). The autoradiographic labeling pattern of (DBE), (DDE) and (DDE) was compared with the pattern of normal adult binocularly (BE) and monocularly (ME) exposed animals. During the experiment the pigeons were moving in an avairy.

In groups DDE, DDE and ME labeling of all known visual structures was asymmetrical and heavier on the side contralateral to the open eye. However, the asymmetry, predominantly in the homolateral area of IHA and HA, was most pronounced in DDE. In DBE, with both eyes free an asymmetry was observed only in IHA and HA whereas the labeling in the side contralateral to the deprived eye was lighter. After binocular exposure of normal adult animals (BE) however the hyperstriatum, receiving input from both eyes, was always symmetrically labeled.

Present results suggest, that the asymmetry of hyperstriatal labeling in experiments with both eyes free (DBE), seems to be more closely related to the system having its origin in the contralateral, deprived eye.

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NEURAL CONNECTIVITY IN ISOLATED GANGLIA OF ADULT HELISOMA: STABILITY AND LABILITY DURING CULTURE. Andrew G. H. Belloch, A. Don Murphy and Stanley B. Kater. Dept. Soc., Univ. of Iowa, Iowa City, Iowa 52242.

Previously we described a system for study of neuronal regeneration in helisoma which involves culture in the hemocoel of host snails, of the buccal mass with attached buccal ganglia and salivary glands (Hufry and Kater, Brain Res. 156, 322-328, 1978). Under these conditions synaptic connections remain stable for many months. Recently we have cultured isolated, i.e. completely axotomised ganglia, and in contrast to ganglia with intact axon tracts, found both physiological and morphological changes. Of particular interest are apparent changes in connectivity, indicated by the disappearance of certain chemical synapses, whereas electrical synapses remain stable.

To date we have concentrated on the Protractor Motoneurons (PMB's), a group of cells that receive IFSP's from a coupled network of premotor neurons, the Cyberhorns. In ganglia cultured for one week, spontaneous IFSP's are no longer apparent in most PMB's and have been replaced by IPSP's. In one pair of PMB's the IPSP is present, but is diminished both in amplitude and duration.

In normal ganglia the IFSP may be assayed by Esophageal Trunk (ET) stimulation, owing to the presence of Cyberhorn axons in this nerve. On blocking chemical synapses with high Mg2+ O Ca2+ saline, ET stimulation evokes a barrage of electrical EPSP's in PMB's. These are normally abolished by the chemical IFSP.

ET stimulation of ganglia cultured for one week produces an EPSP barrage in PMB's. These EPSP's are voltage insensitive, are not accompanied by any apparent change of membrane conductance, and gpe therefore thought to be electrical in origin. High Mg2+ O Ca2+ saline increases the amplitude of the EPSP's to increase, presumably by removal of a shunt due to remaining IPSP's.

After two or more weeks of in vivo culture the IPSP of some PMB's was apparently restored to normal. However, such ganglia often become wrapped in a dense neuma which makes intracellular recording impossible.

The temporary loss of efficacy at some chemical synapses is presumably a response to axotomy, while the neuritic growth and apparent restoration of normal connectivity is a remarkable degree of regulative ability in the adult buccal ganglia.

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EFFECT OF EARLY GUSTATORY NEOCORTICAL LESIONS ON TASTE AVERSION LEARNING IN RATS. Rebecca J. Cabral, Stephen W. Kiefer and John Garcia*. Dept. Psychol., Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

In adult rats, lesions of the gustatory neocortex severely disrupt the ability to associate a taste with illness. However, quite often cortical lesions produced before maturity result in less detrimental effects on some learning tasks.

To investigate plasticity in taste aversion learning rats received bilateral gustatory neocortical lesions (GNC) as neonates (10 days), weanlings (20 days) or adults (70 days). They were allowed to recover and were tested on acquisition and extinction of a saccharin aversion at 120 days of age. For training, the rats were habituated to a drinking schedule in the home cage. They received a preexposure to a 0.1% saccharin solution (day 117), three saccharin-lithium chloride (0.15M, 250 w., 1.p.) acquisition trials (days 120, 123, 126) and five saccharin extinction trials (days 129, 132, 135, 138, 141). After testing, histological identification of the lesions was performed.

The results showed that rats receiving GNC lesions as neonates (ne)10), weanlings (w)17) or adults (a)10) demonstrated significant deficits in acquisition and extinction when compared with controlled, sham-operated or normal control rats. After three acquisition trials GNC rats, regardless of age at lesion, reduced saccharin consumption to 50% of water baseline compared to 100% reduction by control rats.

Thus, sparing of taste aversion learning does not occur when GNC lesions are sustained as early as ten days of age. These results agree with other reports of recovery of function after early cortical lesions.

PLASTICITY

Supported by grants NS 06360 and NS 074447 and Dr. Eric Slack-Gyr-Foundation.
RAFID KINDLING INDUCED BY LOW-FREQUENCY STIMULATION OF THE AMYGDALA. Donald F. Cain and Michael E. Corcoran, Dept. Psychology, Western Ont. London, Canada M5C 1Z2 and Dept. Psychology, U. Victoria, Victoria, Canada V8W 2Y2.

In kindling has suggested that low-frequency stimulation (below approximately 5 Hz) is ineffective in kindling amygdaloid seizures. The present experiment reassessed this question using bi-phasic square wave pulses of 1.0 msec duration delivered at 2.0 or 3.0 Hz through bipolar electrodes chronically implanted into the basilar pontine gray. The present experiment reassessed this question using bi­
phasic square wave pulses of 1.0 msec duration delivered at 2.0 or 3.0 Hz through bipolar electrodes chronically implanted into the basilar pontine gray. The course of 8 experimental sessions over a 3 week period in one monkey, the duration of this enhancement was extended to at least 5 days. On subsequent testing and observation show that maximum response amplitude is not simply related to "arousal" for continuous tail pinch is ineffective. Rather, the excitability of this CM-Pf complex appears related to the animal's exploratory and attending to environmental events. The CM-PF potential is elicited by general anesthesia with ketamine, thiamylal or chloralose and markedly reduced by being replaced by mor­phine. The effect of mor­phine is reversed by naloxone (0.1 mg/kg I.M.) reverses the effect of morphine and unmasks the stimulus-induced enhancement blocked by morphine.

A 4 Hz peak latency potential is recorded bilaterally with maximum amplitude in the centre-medial parafascicular complex (CM-PF) of the medial thalamus. This potential is unaffected by repetitive stimulation but shows a second to second variation in amplitude (from 100 µV to 2000 µV) in the awake animals. Repeated testing and observation show that the maximum response amplitude is not simply related to "arousal" for continuous tail pinch is ineffective. Rather, the excitability of this CM-Pf complex appears related to the animal's exploratory and attending to environmental events. The CM-PF potential is elicited by general anesthesia with ketamine, thiamylal, chloralose and markedly reduced by being replaced by morphine. The effect of morphine is reversed by naloxone (0.1 mg/kg I.M.).

These findings demonstrate the existence of opiate and anesthesia-sensitive pathways which differentially affect different thalamic regions and are likely to play an important role in the selective acquisition and storage of information. The results further emphasize that unilateral, unanesthetized animals in neurophysiological investigations of reticulo-thalamic systems.

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visual areas of the same brains appears normal in distribution and density.

The configuration of callosal degeneration in the occipital region following total section of callosal fibers has been described previously for normal adult albino and pigmented rats (Osiek, 1978; Neurosci. Abstr.). We have examined the consequences of the following experimental manipulations on the developing visual callosal pathway: 1) dark rearing, 2) unilateral enucleation, 3) bilateral enucleation and 4) unilateral section of the corpus callosum were sectioned by a paramedian approach. Lesions were filled more extensively with Vultex or Rockland liquid photographic emulsion than those in grade 3-4 rats. The lesions were filled more extensively with Vultex or Rockland liquid photographic emulsion than those in grade 3-4 rats.

Animals were reared in the dark from birth or were operated on the day of birth. At 4-8 weeks of age the fibers of the corpus callosum were sectioned by a paramedian approach. Rats were performed ipsilateral to unilateral enucleation and contralateral to the tract lesions. After a 2-6-day survival, the animals were perfused and the brains processed by the Fink-Heimer method. Brains were sectioned coronally, or the unlesioned hemisphere was flipped during fixation, sectioned tangentially and prepared by a bleached Fink-Heimer technique.

Dark rearing and unilateral enucleation have little appreciable effect on the configuration of the callosal projection, its density, or the breadth of its distribution. However, bilateral enucleation and optic tract section produce very pronounced changes. After bilateral enucleation, the normal callosal pattern is recognizable, but additional bands and rings of callosal input lie within normally callosal regions of areas 17 and 18. The spread of degeneration into area 17 is prominent laterally and posteriorly. Anteriorly, it virtually fills in area 17. In contrast, the callosal projection to the border in the hemisphere ipsilateral to the neonatal tract lesion is severely decreased in width relative to normal. This effect is specific to the visual callosal system, since the callosal input to the visual areas of the same brains appears normal in distribution and density.

Comparison of the results from albino and pigmented rats and the findings in dark reared and unilaterally enucleated animals shows that in cases in which an intact but abnormal visual input is present, the callosal projection to the hemisphere ipsilateral to the neonatal tract lesion is severely decreased in width relative to normal. The results from similar experiments in cats. However, after neonatal bilateral enucleation, optic tract section, or thalamotomy (Osiek, 1978; Neurosci. Abstr.), lesions in which the corpus callosum is totally deprived of a retinal input via the ipsilateral geniculocalcarine nucleus, the callosal pathway is abnormal.

Supported by USPHS grants NS-07656 and GM-07108 from the National Institutes of Health.)

**PLASTICITY**


Occlusion or transection of the middle cerebral artery (MCA) in the Wistar rat is followed by expansion of the collateral arterial supply vessels and MCA field tissue survival (Neurosci. Abstr. 4: 469, 1978). In contrast, for spontaneously hypertensive stroke-prone rat (SHRSP) the procedure results in tight endothelial cell junctions opening, Evans blue-albumin extravasation, reduced arteriolar filling, tissue necrosis and atrophy extending from the occlusion site to the anatomic zone (Anat. Rec. 193: 740, 1979). Objectives were to evaluate the response in SHRSP following ipsilateral carotid artery occlusions and/or cervical sympathectomies. Twenty-one SHRSPs ranging in age from 33 to 330 days and of either sex were anesthetized intramuscularly with ketamine hydrochloride, 125-200 mg/kg body wt. In 6 animals the right superior cervical ganglion was excised or the cervical sympathetic trunk was transected from the common carotid artery then the artery and its branches was completely ligated and cut 15-20 days prior to the MCA experiment. In the remaining rats, the nerves were carefully dissected from the common carotid artery then the nerve and its external branches were each double ligated and cut 15-20 days prior to the transaction of the right MCA just dorsal to the rhinal fissure. To mark opened endothelial cell tight junctions and tissue necrosis, 1 ml of Evans Blue in physiologic saline was injected intravenously immediately before the MCA operation. Six rats died 3-5 days later, 8 had Horner's Syndrome. Lesion size was graded 1-4 with 4 extending from the transaction site to the zone of following. Total side MCA rats showed grade 3 and 4 lesions whereas 6 intermediate age SHRSPs (66-86 day) had lesions graded 1 or 2. Lesion grade was not related to the asymmetries. MCA vessels in rats with 1-2 lesions were filled more extensively with Vultex or Rockland liquid photographic emulsion than those in grade 3-4 rats. The data indicate cortical occlusions 5-6 days prior to the MCA experiment facilitate the collateral expansion, possibly due to a lowered blood pressure.

Supported by a grant from the Michigan Heart Association.

**2119 THE EFFECTS OF BILATERAL SEZERATIUM VERUS SINGLE-STANCE HIPPOCAMPECTOMY ON THE ACQUISITION OF DRL 20 IN JUVENILE, ADULT AND AGED RATS.** S.D. Gheusi and A. L. Nommensen. Dept. Psychology, Univ. of Kentucky, Lexington, Ky 40506

Previous research has shown that performance on a low rate operant schedule (20 sec CRF) is severely impaired after bilateral single-stance lesions of the hippocampus in adult rats. In contrast, DRL 20 performance is far more affected by hippocampal lesions if they are separated by interoperative DRL training. This study attempted to determine whether this serial lesion effect would also occur in juvenile or aged animals. Rats from each of 3 age groups (15, 160, or 570 days) received training on CRF prior to either single-stance hippocampectomy, sham operative treatment, or bilateral lesion of either anterolateral or posteroventral hippocampus. After forty days of DRL 20 training, the serial lesion groups received bilateral lesions of remaining hippocampus. All animals then received thirty final DRL training. Relative to the single-stance hippocampectomy, serially lesioned animals of all ages demonstrated substantial recovery of DRL 20.

Also addressed in this study were 1) the nature of the 'recovered' behavior and 2) the generalizability of the 'recovery' across multiple cue box movements of each animal while performing DRL and the latter by a final shift back to CRF. Both of these measures indicated that the serially lesioned 'recovered' animals were still very much like the single-stance operates (i.e. they tended to show less variety of operant box movements than controls and they shifted to CRF more slowly than controls).

The facts that 1) all three ages of serially lesioned animals demonstrated similar DRL capacities and 2) the 'recovery' was apparent test specific, both serve to reject a recovery phenomenon that does not involve partial reestablishment of brain connectivity (a capacity that is apparently age dependent). Rather a learning mechanism is proposed that is in essence an application of Ramati's multiple cue blocking theory. Specifically, it is proposed that during a serial lesion sequence, the subtotal disruption of a neural system that exists during the interoperative period allows only subsymptomatic performance and therefore forces a recognition and recruitment of redundant, normally non-preferred cues. Following the second lesion these now 'primed,' nonpreferred cues serve to mediate the behavioral.


The configuration of callosal degeneration in the occipital region following total section of callosal fibers has been described previously for normal adult albino and pigmented rats (Osiek, 1978, Neurosci. Abstr.). We have examined the consequences of the following experimental manipulations on the developing visual callosal pathway: 1) dark rearing, 2) unilateral enucleation, 3) bilateral enucleation and 4) unilateral section of the corpus callosum were sectioned by a paramedian approach. Six rats died 3-5 days later, 8 had Horner's Syndrome. Lesion size was graded 1-4 with 4 extending from the transaction site to the zone of following. Total side MCA rats showed grade 3 and 4 lesions whereas 6 intermediate age SHRSPs (66-86 day) had lesions graded 1 or 2. Lesion grade was not related to the asymmetries. MCA vessels in rats with 1-2 lesions were filled more extensively with Vultex or Rockland liquid photographic emulsion than those in grade 3-4 rats. The data indicate cortical occlusions 5-6 days prior to the MCA experiment facilitate the collateral expansion, possibly due to a lowered blood pressure.

Supported by a grant from the Michigan Heart Association.

**2121 RECOVERY FROM UNILATERAL NEGLECT: BEHAVIORAL AND FUNCTIONAL ANATOMIC CORRELATIONS IN MONKEY.** Edmund M. Diamant, Robert C. Collings, Nancy Dunlop* and Torris V. Caston*. Departments of Pediatrics & Neurology, Washington University, St. Louis MO 63110.

Unilateral damage to frontal cortex in the periscruate region (PPC) in monkeys results in a syndrom of contralateral visual and tactile neglect and an absolute preference for the ipsilateral hand. Spontaneous, complete recovery occurs by unknown mechanism. We have used the [3-14C]-deoxyglucose autoradiographic technique (DG) to examine the regional cerebral glucose utilization during three phases of recovery. Five adolescent Macaca fascicularis were tested with a standard neurological exam and taught a supination-supination motor response to criterion before operation. PPC was removed by subpial resection from the right hemisphere, testing was repeated postoperatively, and animals were sacrificed 7 days after the last test. The brains were perfused with formalin, frozen in freon at ~50°C, then cut at ~30°C. Four adjacent 30 μm sections were taken at 600 μm intervals through the entire brain (three for densitometric analysis, the other for histologic study).

Animals examined within two weeks after injury showed severe contralateral neglect, including homanopia and absolute preferrence for the hand ipsilateral to the lesion, and no retention of the conditional motor response. DG studies showed a 10-40% depression of DG uptake in ipsilateral (Right) subcortical structures, primarily thalamus and basal ganglia. Compared to contralateral (Left) homotopic sites, the greatest depression of DG uptake occurred in n. medialis dorsalis (Right = 54% of Left), n. reticularis (R = 70% L), n. ventralis anterior (R = 74% L), n. X of Olszewski (R = 75% L), caudate (R = 75% L), putamen (R = 77% L), and pulvinar (R = 80% L). Animals showed substan­
tial recovery from the lesion by four weeks. DG studies at the time revealed only a slight depression of glucose uptake (~10%) in ipsilateral subcortical sites, principally in caudate (R = 93% L) and putamen (R = 92% L). In these cases the three phases of depression of ipsilateral cortical DG marked.

The findings lead us to postulate that the multimodal neglect is related to a widespread metabolic dysfunction in subcortical territories. The monkeys' recovery from neglect occurs in parallel with reappearance of symmetrical metabolic activity in these structures.
GOLGI ARCHITECTONICS OF A REGION INVOLVED IN SONG PRODUCTION IN THE CANARY. T. J. DeVries and F. Nottebohm. Rockefeller University, New York, N. Y. 10021.

Nucleus robustus archistriatalis (RA) is a large telencephalic nucleus in the canary. When RA is lesioned in singing male canaries, song is disrupted (Nottebohm et al., J. Comp. Neurol. 165:457, 1976). RA is distinguished by the propagation rate of antidromic spikes elicited in the postganglionic nerve trunk. The two types of ganglion cells are selectively innervated by separate populations of preganglionic axons; B-fibers innervate B-cells, and enter the tectum at the seventh and eighth segmental levels. Thus, fast excitation reinnervated by B-fibers. Approximately 16% of reinnervated C-cells (n=48) has never been observed, and B-fiber input has been observed in approximately 4% of C-cells (n=48). When examined 6-22 weeks after crush of both B- and C-fibers, most ganglion cells were reinnervated. From a total of 188 B-cells examined, only 2 were reinnervated by C-fibers, while most were appropriately reinnervated by B-fibers. Approximately 16% of reinnervated C-cells, or 12% of the total C-cell population (n=146), were reinnervated by B-fibers, while most were appropriately reinnervated by C-fibers. Thus, while regenerating preganglionic fibers occasionally form synapses with inappropriate target cells, reinnervation is selective on the whole highly specific when assessed at relatively long times after denervation. Preliminary observations 3-4 months postoperatively suggest that during early reinnervation, there is a higher incidence of inappropriate connections: 75% of reinnervated C-cells (66% of all C-cells) in 4 preparations had been reinnervated by B-fibers. No B-cells innervated by C-fibers were observed.

These results indicate that the selective innervation of B- and C-cells which exists in normal adult frogs is restored upon regeneration of the cut regenerating fibers by a combination of accuracy in restoration of contacts and elimination of inappropriate connections which do arise. Supported by N.I.H. Grant NS 10792.

LONG-LASTING POTENTIATION IN THE PERFORANT PATHWAY TO CA1 NEURONS IN THE HIPPOCAMPUS, IN VITRO. Herbert J. Pollela and Forrest F. Heimer. Depts. of Physiology S Neurobiology, Boston University School of Medicine, Preclinical Studies, National Institute on Alcohol Abuse & Alcoholism, Rockville, Md. 20852.

In the hippocampus several pathways have been shown to exhibit a long-lasting potentiation following a brief tetanic stimulation of the pathway investigated. This potentiation of evoked responses has been described in vitro in CA3 preparations (Brain Res. 89:107, 1975) and has been reported to last for days in whole animal preparations (J. Physiol. 232:137, 1973). Anatomically, the perforant pathway terminates, in part, on the apical dendrites of the CA1 pyramidal neurons. We recently reported electrophysiological evidence for this pathway in the hippocampal slice preparation (Soc. Neurosci. Abst. #679, 1978). We report here a long-lasting potentiation of this perforant to CA1 pathway in the hippocampal slice.

Male Hartley guinea pigs weighing 300 to 400 gm were sacrificed. The hippocampus was quickly removed and placed in ice-cold medium. Thin (350 to 400 µm) transverse sections of the hippocampus were cut using a Sorvall tissue sectioner. Using small knives, a section (1 to 2 mm wide) in the CA1 region of the slice was removed. This section started at the ventricular surface and included regions down to but not including perforant pathway. Removal of this section insured that CA1 activation was not the result of stimulating other pathways. The section was transferred to a trough-like chamber and superfused at a rate of 5 mI/min with medium saturated with 95% O2 & 5% CO2. The medium contained 124 mEq NaCl, 3.8 mM KCl, 2.8 mM CaCl2, 1.24 mM NaH2PO4, 10 mM glucose, and 2.6 mM NaHCO3. A bipolar stimulating electrode was placed in the perforant pathway on the entorhinal side of the hippocampal slice. The pathway was stimulated at a frequency of 1/min with pulses of 0.02 msec duration and amplitudes of 10 - 100 V. To produce potentiation, a tetanus of 15 sec was used. The maximum size of the field potential was determined by measuring from the maximum negative peak of the population spike to the peak of the positive wave following the field potential. Following the tetanus, the amplitude of the field potential increased 150% to 300% and remained elevated for at least 15 min. These data indicate that CA1 neurons exhibit long-lasting potentiation in response to perforant pathway stimulation.


In frog lumbar sympathetic ganglia, B-cells and C-cells can be distinguished by the propagation rate of antidromic spikes elicited in the postganglionic nerve trunk. The two types of ganglion cells are selectively innervated by separate populations of preganglionic axons; B-fibers innervate B-cells, and enter the tectum at the seventh and eighth segmental ganglia. These results indicate that the selective innervation of B-cells is achieved by a combination of accuracy in restoration of contacts and elimination of inappropriate connections which do arise. Supported by N.I.H. Grant NS 10792.
Two forms of sprouting are present in the neonatally deafferented dentate gyrus of the rat. Christine Gall and Gary Lynch. Dept. of Psychobiology, UCI, Irvine, Ca.

The collateral sprouts of the dentate gyrus of the rat, normally restricted to the inner one third of the dendritic field, sprout into the more distal region following deafferentation of the dentate gyrus induced by ipsilateral hemispherectomy. Previous autoradiographic and electron microscopic studies have demonstrated that following such lesion placement in the 14 day old rat the commissural sprouting exhibited by the dentate afferents rapidly expanded to occupy the full depth of the dendritic field and ultimately establish an even density of synapses throughout. In the present study this postlesion response was reexamined using Holmes stain for normal fibers.

In those pups sacrificed 4 days after the lesion, the dentate's inner molecular layer fiber plexus (known in the adult rat to correspond with the commissural and associational afferent systems) was not found to be expanded; a surprising observation in light of autoradiographic data demonstrating that the commissural system has in fact sprouted by this postlesion interval. However, by 6 days postlesion the plexus was enlarged to approximately the same extent as seen after entorhinal lesions in the adult. With longer postlesion survival this plexus expands no further relative to controls leaving the most distal aspects of the deafferented dendritic field remarkable fiber-poor. In addition to this delayed and limited plexus expansion the appearance of a few unusually large caliber axons in the most distal deafferented field was noted, being particularly evident at the longer survival intervals.

The present data, considered in light of the previous autoradiographic and electron microscopic work and the present findings, suggest that two forms of sprouting are present in the neonatal hippocampus: one being rapid and extensive as described by the autoradiographic and electron microscopic data and the second being more limited and exhibiting a several day postlesion delay to onset. This latter form observes the same spatial and temporal parameters as exhibited by the normal commissural sprouting induced by entorhinal cortical removal in the adult rat. It is proposed that the former more extensive form of growth is exhibited by only a small subset of axons, and that this form of sprouting is lost to unmask with development the more limited pattern of adjustment. This second more restricted form of sprouting is then retained in the adult. (Supported by NSF grant BMS76-17370 to G.L.)

The brain stem auditory system of chickens provides an opportunity to predict specific cellular changes in central neurons that may result from differences in sensory experience. The dorsal and ventral dendritic endbranches of nucleus laminaris (NL), a third-order brain stem auditory nucleus, receive segregated excitatory inputs from the ipsilateral and contralateral auditory nerves respectively, through a direct contralateral projection system in nucleus magnocellularis (MC). Neuronal activity in the 8th nerve, HM and NL monotonically increases with increasing acoustic stimulation. Silicone earplug devices that provide a 40 db broadband conductive hearing loss were placed in one ear of embryonic chickens before they entered the air space of their eggs (day E 18) and were maintained in place for 28 days thereafter. At 25 days post-hatch, the brains were coded and then impregnated by a Golgi-Kopsch method. Six to ten clearly impregnated NL cells from each side of each chick were paired by position in the nucleus and drawn under camera-lucida. Blind procedures were used to prevent observer bias. The number of primary dendrites and the total projected length of the dendrites were measured on the dorsal and ventral side of every cell that had been drawn. In every bilateral pairing of cells, the dorsal and ventral dendrites that received input from the same ear were compared with the dorsal and ventral dendrites from those same cells that received input from the deprived ear.

Analysis revealed that a statistically reliable decrease (p <.01) in the number of primary dendrites on the sides of the NL cells receiving input from the deprived ear. An average loss of 14% of the primary dendrites on the deprived sides of the cells was observed. A small decrease in the projected length of the deprived dendrites, that was not statistically significant, was also observed.

In conclusion, a unilateral change in sensory experience differentially affected the form of specific dendritic surfaces of central neurons receiving input from the same ear. This change was predictable from the strength and pattern of innervation of those neurons. These results are consistent with the hypothesis that local synaptic activity can influence the form of post synaptic elements. (Supported by NSF grant BMS76-48074, funds from the deafness Research Foundation and the Sloan Foundation, and NIH grant AM-053490)

In conclusion, a unilateral change in sensory experience differentially affected the form of specific dendritic surfaces of central neurons receiving input from the same ear. This change was predictable from the strength and pattern of innervation of those neurons. These results are consistent with the hypothesis that local synaptic activity can influence the form of post synaptic elements. (Supported by NSF grant BMS76-48074, funds from the deafness Research Foundation and the Sloan Foundation, and NIH grant AM-053490)
CHARACTERIZATION OF IDENTIFIED NEURONS OF HELISOMA MAINTAINED IN IN VITRO ORGAN CULTURE FOR THE STUDY OF REGENERATION.


Previous studies using an in vivo organ culture have shown that regeneration of the central nervous system can functionally regenerate and specifically reinnervate postsynaptic target organs subsequent to axon interruption (Murphy and Kater, 1978). We are developing an in vitro culture method for Helisoma tissues using media based on commercial M199 (GIBCO) medium with salts adjusted to Helisoma hemolymph values.

Buccal muscles and muscles characteristic of feeding behavior in vivo are observed for at least two weeks in culture. Intracellular recording and staining techniques show that the physiology and morphology of these preparations differ little from normal preparations. Overshooting action potentials and synaptic inputs to specific neurons in vitro have the same characteristics as neurons in non-cultured preparations.

Earlier studies have shown (with in vivo culture) that following a nerve crush, axons of salivary secretoeffector neurons extend many sprouts through the crush site and reach the salivary glands in less than one week. Present in vitro studies show that these neurons, when axotomized, extend sprouts beyond the crush site in a similar length of time, though the exact time course of growth is variable. Furthermore, spraying in vitro occurs by exactly the same mechanism as regeneration.

That is, growth occurs by the extension of numerous small sprouts from the injured axon and these sprouts extend through and beyond the site of nerve trunk injury. Finally, in agreement with previous findings, the presence of a viable postsynaptic target organ is necessary for initiation of sprouting.

Sprouting of injured neurons can occur in vitro in a preparation consisting of only the buccal ganglia and buccal musculature. Observations on growing neurons in vitro are quite comparable to regeneration in vivo and allow us to employ in vitro organ culture as a higher resolution approach to questions of neuronal pathfinding.

Supported by MH 13172 (R01) and RO1 NS09696 (R01). REFERENCE

PLASTICITY


This is part of a quantitative study of turnover of central catecholaminergic (CA) elements under experimental conditions. The tendency of boutons to reaccumulate within the paraventricular hypothalamic nucleus (PAR) subsequent to neurotoxin lesions is a noteworthy one. We have determined CA fluorescence microscropy and electron microscopy. CA terminals in the PAR were further characterized by a high proportion of synaptic contacts. The paraventricular nucleus therefore provides an opportunity to assess the impact and limits of the sprouting responses. Consequently, the synapses are in a functional state. Data about bouton sizes and numbers of synapses will be dealt with separately.

Young adult male rats were perfused and the hypothalamus prepared for fluorescence microscopy. The terminals identified were counted, and the size of individual boutons measured. A total of 12,000 boutons were counted with a Zeiss microscope. CA terminals in control preparations. Synapses were evident around 31% of boutons A 21 days after the neurotoxin. These results suggest that the new CA sprouts (seen 21, 56 and 180 days post-lesion) are associated with synaptic specialization.

(ML2194 to JU; NS1650 to THM.)


We found previously in rats that intact low-threshold mechanosensory nerves appeared to sprout into adjacent denervated skin only prior to about 20 days of age; in older animals this "denervation sprouting" did not occur (Jackson and Diamond, Abstr., 1511, Soc. Neuroneuro., 1978). We have now evidence that high-threshold mechanosensory nerves can be induced to sprout in denervated adult rat skin. In these experiments the presence of high-threshold fibers was defined in vivo by their ability to anesthetized rats, by the ability of brief forceps-pinches of back skin to elicit a reflex contraction of the underlying cutaneous truncal muscle. In three groups of adult animals clearly defined areas of back skin lacking sensitivity were selected by section of selected dorsal cutaneous nerves. The first group was behaviourally mapped one day after operation. The second group was mapped 24 days after operation. The animals of the third group were lightly anesthetized at 4 day intervals, and pinches (somewhat stronger than those used for the behavioural testing) were applied to both innervated and denervated skin regions; at 24 days after the denervation behavioural mapping was done.

Results from the first two groups showed that in the 24 days following the denervation there was no significant shrinkage of the insensitive regions (675 ± 140 (mean ± S.D.) mm², and 625 ± 150 mm² respectively). The results of high-threshold nerve sprouting. In the third group however the insensitive skin regions shrank (to 215 ± 150 mm²). It would appear then that those axons which sprouted in the first animal were not caused by the extension of the low-threshold "touch" fields, suggesting that regeneration after damage to nerve endings may not be required for the denervation sprouting. Among the possibilities we are now examining is that the combination of nerve-free target tissue plus activity in maintaining nerve functions causes collateral sprouting in the adult animal.

Supported by the Multiple Sclerosis Society of Canada.


Cerebral cortex from rat embryos survives and matures after transplantation to newborn rat brains. We studied the question whether transplants form connections with the host brain and if so, whether these connections were specific for the type of brain region transplanted or whether they were influenced by implant position within the host brain.

Cerebral cortex from rat embryos of gestational age 16 to 17 days was transplanted to the tectal region of newborn rats and allowed to mature for periods of 4 to 7 weeks. Subsequently, efferent fibers to the transplant could be shown by retrograde transport of horseradish peroxidase. Animals with transplants that were either embedded in the host tectum or connected to it by a recognizable fiber trunk were selected for this study.

Small bundles of efferent fibers could be demonstrated most often in the central gray and superior colliculus of the host. In some cases distribution of transplant afferents was noted in the pretectum and midbrain tegmentum. Afferent connections to cortex transplants were identified from layer V pyramids in various regions of the host cortex, pretectal neurons, and occasional neurons from deep layers of the host superior colliculus. The afferent fibers to cortex transplants are relatively sparse compared to afferent connections that are formed if embryonic tectum is transplanted to the same region. This phenomenon, in other experiments, in the same host tested could retinal afferents be demonstrated within the cortex transplant.

The results indicate that cortex transplants develop fiber connections with the host brain. For the lack of retinal afferents the pattern of connections formed more closely resemble those expected of superior colliculus rather than cerebral cortex. This suggests that location is a significant factor in determining fiber connections which form between the transplant and host. However, qualitative and quantitative differences in transplanted-host fiber connections could be observed in cortex transplants, compared to tectal or retinal transplants placed in a similar position of the host brain. Consequently, the influence of neuron specific qualities on pathway formation cannot be disregarded. (Supported by USPHS Grant EM-01950 from the NIH.)

2137 EFFECTS OF DIFFERENTIAL POSTWEANING ENVIRONMENTS ON DENDRITIC FIELDS OF MALE AND FEMALE RATS. Janice M. Myers and William T. Greenough. Dept. Psychol. and Neural & Behav. Biol. Prog., Univ. of Ill., Champaign, Ill. 61820.

There is behavioral evidence that females are less suscepti­ble to the effects of development than males (e.g., Sackett, Sex Differences in Behavior, R. C. Friedman et al. (eds.), 1974). There is also neuroanatomical evi­dence that female rats, when exposed to a complex environment from 60 to 116 days of age, show a smaller change in visual cortex thickness than do male rats (Diamond et al., Intern. J. Neurosci. 2:171, 1974). In the present experiments, resting fields were examined after male and female rats had been exposed to differential environments at weaning. Twelve littermate sets of two male and two female hooded rats were assigned to either a complex environment (CE) with other same sex rats and daily toy changes or to an isolated standard laboratory cage (UC) at weaning (23-25 days of age). After 30 days of environmental exposure, the brains of all animals were stained with the Golgi-Cox method and 100 mm coronal sections were taken from the vis­ual cortex area. Fifteen layer IV stellate neurons were traced with the aid of a camera lucida from each number of seven litters of each sex. Each neuron was scored for number and length of dendritic branches and the number of intersections of branches with a overlay of concentric rings at 20 micron intervals. Although there were no differences in the number of dendritic branches, the dendritic fields of both sexes may respond to differential environments but that the male response is somewhat greater.

Supported by MH 07286 and NSF BNS 7723660.
2138 REDIRECTION OF PYRAMIDAL TRACT AXONS IN NEONATAL HAMSTERS: SOME MECHANISMS OF AXIAL GUIDANCE DURING DEVELOPMENT. K. Kallì and T. Reh. Dept. of Anatomy and Neurosciences Training Program, Univ. of Wis., Madison, WI 53706.

In previous experiments we have shown that when pyramidal tract axons are regrown in neonatal hamsters there is massive regrowth of the severed axons via a new brainstem pathway which decussates in an aberrant position, descends through the brainstem in an abnormal course, but nevertheless terminates appropriately in the correct spinal cord column and spinothalamic tract of the correct side of the brain (Kallì and Reh, ’76). Regrowth of the cut fibers is maximal in animals 4–6 days of age.

In the present experiments, animals younger than 48 hours received a unilateral lesion of the medullary pyramid several mm rostral to the decussation of the tract. At this age a substantial portion of the fibers are still growing into the medullary pyramid. Thus when (H)³ prolinc was injected into the ipsilateral sensorimotor cortex of animals surviving to adulthood, we observed not only regrowing fibers as previously described, but also many apparently later arriving fibers which were redirected rather than damaged by the lesion. Many of the fibers were deflected across the midline and descended through the intact contralateral pyramid. Surprisingly, when these fibers reached the decussation they recrossed to the “wrong” side of the brain. Other fibers which were deflected completely away from both pyramids descended through the brainstem to form a completely anomalous pathway in the lateral margin of the spinal cord. These findings suggest that (1) Growing axons deflected across the midline to the opposite side of the brain appear to be pulled along to the incorrect side by the decussating normal fibers; (2) Axons deflected far from the decussation on the side of the brain rather than crossing; (3) Growing axons appear able to descend through channels or pathways which under normal circumstances they never follow. These results indicate that mechanical factors in the substrate rather than intrinsic factors in the axon itself may play a more important role in determining specificity than intrinsic factors in the neurons from the intact pyramid, (2) Axons deflected far from the decussation on the side of the brain rather than crossing, (3) Growing axons appear able to descend through channels or pathways which under normal circumstances they never follow. These results indicate that mechanical factors in the substrate rather than intrinsic factors in the axon itself may play a more important role in determining specificity than intrinsic factors in the axons themselves.

SOME MECHANISMS OF AXONAL GUIDANCE DURING DEVELOPMENT. (Supported by NSF grant NS-14428 and NHI training grant GM 07507.)


It has been shown that the effect of catecholamines (CA) on the growth and plasticity of visual cortex is due to their terminals in the suprasylvian plasticity in the kitten visual cortex. In the previous physiological study, we used 14-C-6-hydroxydopamine (6-OHDA) to reach a region about 5 mm away from the perfusion site after a week-long, continuous microperfusion (Kasamatsu et al., J. Comp. Neurol. 185, 1979). In the present study, we have microfluorometrically examined the intracortical distribution of tritiated NE and norepinephrine (NE) delivered through the continuous perfusion technique. Three methods have been used.

1) CA histofluorescence.

The kitten visual cortex was perfused locally with 4 mM 6-OHDA for a week, and then prepared for CA histofluorescence using a modified glycylglycine- cryostat method. We found an area close to the perfusion site in which no greenish fluorescent fibers and terminals remained. The radius of this primary lesion area was about 5 mm from the center of perfusion.

2) Endogenous CA.

An electrochemical assay was performed for NE and dopamine (DA) (Keller et al., Life Sci. 19, 1976) in kitten visual cortex after the microperfusion of 6-OHDA. Endogenous NE was less than 10% of the control 3–4 mm away from the perfusion site, and then it increased to 50–80% at 7–8 mm. The DA content was less affected by 6-OHDA treatment.

3) Intracortical distribution of tritiated H

The spatial distribution of tritium in the neocortex was studied following 1, 3, or 7 days of continuous perfusion with 50 µM nonradioactive NE mixed with a tracer quantity of tritiated NE. As reported previously, counts were maximal at the site of perfusion and decreased exponentially in the neighboring area (Kasamatsu et al., J. Comp. Neurol. 185, 1979). This distribution pattern was attained as early as 1 day after the start of perfusion. Although the radioactivity at a given site in the cortex increased with time, its maximum detectable extent was about the same (10–20 mm) for the three different perfusion methods.

Combined with the dose-response relation obtained in other physiological experiments, the above information has enabled us to estimate that the terminal localization for NE’s effect on synaptic plasticity is around 10⁻⁷ M.

(2141 FAILURE TO FIND SPARING OF SPECIES TYPICAL BEHAVIORS FOLLOWING NEONATAL PREFRONTAL CORTEX LESIONS. Brian Bolb and Ian Q. Whishaw. Dept. of Psych., U. of Lethbridge, Canada.

Rats with lesions of the prefrontal cortex (PFC) in infancy exhibit remarkably spared performance compared to adults with similar removals. Lesions of the medial frontal (MF) cortex in infancy fail to produce deficits on tests of cognitive behavior such as spatial reversals, delayed response and active avoidance which are severely disrupted by lesions in adulthood. Furthermore, unlike adult lesions these early lesions fail to produce the degeneration in the dorsomedial nucleus of the thalamus that typically accompanies adult lesions in PFC (Bolb & Nonnenmacher, Brain Res. 156, 151, 153). The findings that early lesions can spare certain functions led to the question: which behaviors are sensitive to neonatal brain injury that are spared by adult lesions? Was the sparing of function being mediated by other frontal cortical zones? This was tested by removing the entire frontal lobes at 7, 25 or 100 days of age. Would there be sparing on such tests of natural behaviors as food hoarding or maternal behavior? This was studied by observing the same animals on tests of spatial reversals, active avoidance, hoarding, and terminal behavior as well as by videotaping each rat's home cage behavior for 24 h.

The results showed that sparing following PFC lesions is task specific. Spared behaviors as food hoarding and maternal behavior were found in the 24 hour home cage study where the 25 and 100 day Ss were normal. Removal of the PFC at 7 or 25 days of age allowed normal performance on spatial reversals and active avoidance but abolished hoarding and maternal behavior. In the 24 hour home cage study the 7 day Ss were virtually identical to controls whereas the 25 and 100 day Ss had difficulties in drinking from a drinking tube and were generally less active.

Anatomically, the lesions in 7 day operated rats were smaller to visual inspection but the brains of these animals were significantly smaller than those of controls. At 25 and 100 days the brains of the operated animals were about 85% of the 25 and 100 day Ss even though there was no difference in body weight. We again failed to find degeneration in the dorso-medial nucleus of the thalamus of the 7 day Ss as compared to the 25 and 100 day animals.

These results imply that species typical behaviors are more sensitive to neonatal brain injury than the more task-specific cognitive behavior. It is likely that other regions of the brain can assume normal functional properties and that the brain injury is not sufficient to disrupt general functions required for acquiring problems such as spatial reversals or the more task-specific behaviors such as those observed in animals with neonatal brain injury.

(Supported by N.I.H. Grant AG-00001)
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2142 PLASTICITY OF A SOMATOSENSORY CORtical COLUMN: A COMPARISON OF THE EFFECTS OF NEONATAL AND ADULT RECEPTOR ABLATIONS IN THE RAT USING THE (14C)-2-DENOXYGLUCOSE TECHNIQUE. M. Kosuke*, P. Hand, J. Greenberg*, A. Sylvestro*, C. Gooch*, and M. Revlish. Departments of Animal Biology and Neurology, Schools of Veterinary Medicine and Medicine, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania 19104; Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, University of Warsaw, Poland, 20014.

Plasticity of cortical representation of mystacial vibrissae of rats (barrel columns in SI cortex) following high frequency stimulation of one whisker was studied by the (14C)-2-deoxyglucose (2DG) method in unanesthetized, restrained rats. The local cerebral metabolic rate of glucose (LCMR) of barrel column corresponding to stimulation of CM vibrissae was determined. The stimulation was brush stroking for 45 minutes preceded by an intravenous injection of 50 uCi of 2DG as described by Hand et al. (Neurosci. Abst., 3:41977). They demonstrated that stimulation of one whisker increases the LCMRG of one cortical column, which extends from layer I to VI in a candle-pin shape.

Columnar plasticity was investigated 3 months after unilateral ablation of all but the CM vibrissal follicle, done either in adulthood or 2 to 4 postnatal days. With adult receptor damage the appearance of the column related to single vibrissal stimulation resembles the control, being easily discernable in all layers. However, LCMRG of layer IV of the column is decreased by 7%, while that of the lower part of lamina V is higher (18%) than in the control column. The diameter of layer IV activation is 205 larger than on the control side, which is in agreement with the barrel measurements of thionin sections. In addition, the activated region surrounding the columnar profile is 1.5 times more extensive in layers I-IV. With neonatal follicle ablations, the cortical area labeled by single whisker stimulation is enlarged and, with the exception of layer IV, the typical columnar profile is lost. The diffuse label within SI cortex is patchy, especially in supragranular layers, unlike the normal column in which the pericolumnar activity gradually diminishes. The LCMRG in layer IV is reduced by 15%, but is elevated in supragranular layers. The diameter of layer IV is also increased (18%) and the pericolumnar activity is much greater in rats with neonatal damage.

In conclusion, these results are in agreement with the findings of electrophysiological alterations observed after neonatal vibrissal removals (Killackey et al., Neurosci. Abst., 3:41977). They demonstrated that selective noradrenergic cell nuclei remain viable after transplantation, and that such nuclei can innervate recipient brain tissue. (Supported by grants NS-06716 of U.S. and 76-10-9 of Sloan Foundation).

2144 ULTRASTRUCTURAL CHANGE IN THE HIPPOCAMPAL SLICE FOLLOWING REPETITIVE ACTIVATION. Kevin S. Lee, Mike Oliver, Frank Scholtie*, Bob Creger, and Gary Lynch. Dept. of Psychology, Univ. of Calif., Irvine, Calif., 92717.

Recently we reported that changes in synaptic structure occur in conjunction with the induction of long-term potentiation (Anat. Rec. 192, 3:601, 1979). Specifically, an increased incidence of synapses onto dendritic shafts was observed in the stratum radiatum of CA-1 in the rat hippocampus following high frequency stimulation of the combined Schaffer collateral and commissural afferents. In the present study we sought to correlate this finding utilizing the in vitro hippocampal slice preparation. This technique has been extensively employed for electrophysiological and biochemical studies; however, little information exists as to its utility for anatomical investigations. Initially parametric analyses were performed to optimize the preservation of neuronal elements. Once conditions for achieving replicable tissue quality were ascertained, experiments were undertaken to examine the effects of repetitive afferent activation on neuronal ultrastructure. Evoked potentials recorded in the stratum radiatum were tested prior to and following either high (200 sec^-1) or low frequency (0.2 sec^-1) repetitive stimulation of the Schaffer collateral and commissural afferent. High frequency stimulation consistently resulted in a non-decremental (over 15 min) increase in the size of the evoked response while low frequency stimulation had little or no effect on evoked response. In the stratum radiatum the synapse on which was activated showed no difference between the high and low frequency stimulated slices in the following measures: 1) the incidence of synapses onto dendritic spines, 2) the incidence of multiple synaptic boutons, 3) spine area, 4) spine stalk width, 5) psd length for synapses onto dendritic spines and 6) psd length for synapses onto dendritic shafts. However, in the potentiated region which was activated showed no difference between the high and low frequency stimulated slices in the following measures: 1) the incidence of synapses onto dendritic shafts was increased by 50% (p<0.01, on a two-tailed test). These results are consistent with the previous data that the only consistent structural modification observed was an increase in the number of dendritic shaft synapses. The primary difference between this and the previous stimulation, after which they were dissected, was the time interval allowed between afferent stimulation and tissue fixation. The slices were fixed for only 15 minutes following stimulation, after which time they were immediately immersed in fixative. It thus appears that the structural change observed can occur at a very rapid rate.

2145 GROWTH OF AXONAL PROCESSES FROM IN VIVO TRANSPLANTS OF RAT LOCUS COERULEUS. Richard M. Kostrzewa and Hideki Fukushina*. Dept. of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, Tennessee 37601.

As a means of determining mechanisms associated with neuronal growth and development, we have transplanted a discrete central noradrenergic nucleus of newborn rats to other neonatal rat brains. Sterile technique was used to remove the locus coeruleus which was then immediately (< 24h) placed into a number of different recipient brain sites. Using a glyoxal acid staining procedure we were able to observe fluorescent cell bodies of the donor cells within the cerebral cortex or neocortex. Cell bodies of the transplant retained their fluorescence, and axonal processes began to emerge from the transplant into recipient brain tissue within 2 days. Using a trihydroxyindole fluorimetric assay, we also have found that the norepinephrine content of the cerebellum is elevated at 2 months after transplantation into this region. These findings indicate that selective noradrenergic cell nuclei remain viable after transplantation, and that such nuclei can innervate recipient brain tissue. (This study was supported by NIH grant no. NS-14797).


In previous work we have shown that by 30 days following unilateral bulbectomy in neonatal mice, olfactory fibers regenerate and penetrate various regions of the ipsilateral telencephalon. Here they form glomerular structures and synapse with neurons within the host forebrain.

Recently, however, we have encountered a number of preparations with long-term survivals displaying a heretofore unobserved degree of neuronal reorganization. Each of these animals was bilobecutized at 5 days postnatally, sacrificed 180-365 days later, and prepared routinely for light microscopy. Our histological results, in general, indicated the typical regenerative phenomena previously noted, i.e., innervation of the ipsilateral telencephalon and glomerular formation in the forebrain by the reconstituted olfactory axon terminals. However, in some animals, we have observed an additional expansion of the intact, unoperated bulb into the space vacated by the operated bulb. Furthermore, many of the regenerated axons on the operated side could be seen entering the fiber plexus innervating the intact bulb. This outgrowth of the intact olfactory bulb may be characteristic of all its cellular components, although it is most strikingly displayed by the granule cells. In one case, the expansion and reorganization of the intact bulb was rather extensive, so that the bulb appeared to be continuous with both the ipsilateral and contralateral telencephalic hemispheres. The role of possible injury to the intact bulb and/or neighboring bone and the pattern of bulbular reorganization is presently under study.

Both the observed bulbar outgrowth and the subsequent innervation of regenerated fibers may result in non-specific connections within the olfactory system and illustrate a potential capacity for additional plasticity after surgical insult to this system. Supported by NIH Grants 5T32 NS07010 (R.L.) and NS 08943 (P.F.C.G.) and NSF Grant BNS 77/16737 (P.F.C.G.).
CORTICAL ORGANIZATION IN PRENATAL ENCEPHALOPLASTIC PORENCHEMY.
A GOLGI STUDY. M. Marin-Padilla and M.T. Marin-Padilla*. Dept.
Pathology, Dartmouth Medical School, N.H. (2146)

Prenatal encephaloplastic porencephalic are characterized by focal
destruction of the cerebral wall which may be reduced to a thin
membrane composed of ependymal and arachnoidal elements,
by hydrocephalus and by glial and vascular scar formation. It is
believed that this type of congenital malformation is caused by an
accidental act of underdevelopment (vascular or infectious) which
resulted in cortical destruction, secondary hydrocephalus and
eventual formation of porencephalic cysts. The fundamental
plan of neuronal migration and cortical organization is consid­
ered to be permanently altered in this disorder. Therefore,
the cerebral cortex away from the cystic lesions may appear to be
histologically normal, or without obvious architectural
abnormalities. On the other hand, areas closer to the defects
display glial and vascular scars, prominent cytologic anomalies and
various degrees of cortical atrophy. Intermediate cortical areas
epidemic, however, more subtle changes including: a tendency
of neurons to form columnar aggregates in upper layers which alternate
with cell-free spaces, arachnoidal glial and vascular scars and the presence of abnormal large neurons in layer II and III.

Rapid Golgi preparations of the cerebral cortex of a premature
infant with this type of disorder has permitted, for the first

COOPERATIVITY IN THE GENERATION OF POST-TETANIC SYNAPTIC ENHANCE­
MENT APPEARS TO REQUIRE NEAR SIMULTANEITY OF APPERT INPUT
B. L. McNaughton and C. A. Barnes. Dept. Psych. Dalhousie Univ.,
Halifax, Nova Scotia, CANADA, N S 1OJ (2149)

Following brief episodes of high frequency activity, synapses of the perforant pathway may undergo a prolonged enhancement of their efficacy (Milis & Lemo, J. Physiol., 1971, 215, 331-346). This enhancement has been shown to involve three cooperativity effects or synaptic enhancement of post-tetanic synaptic enhancement by cocactive afferent fibres (McNaughton, Douglas, & Goddard, Brain
Res., 1970, 221, 277-295). In particular, synapses of the medial and lateral perforant paths to the dentate nucleus in adult cats were shown to exhibit a prolonged enhancement of post-tetanic synaptic enhancement by cocactive afferent input, or whether there might still be some cooperation observed when temporal delays between the bursts of activity on the two input pathways are introduced. This question is of interest not only because of its relation to the mechanism of enhancement, but also because it relates to models of associa­tive memory which postulate enhancement as an underlying mech­anism.

Methods for extracellular recording synaptic responses of the medial and lateral perforant pathways were as described by McNaughton et al. (1976). The experimental paradigm was also essentially identical except for the stimulation parameters. In one hemisphere, 20 stimulus trains of 10 msec at 250 Hz were delivered at alternating square-wave burst to the other and the superficial layers of the host's tectum. It was common for transplants to be embedded in the anterior cerebral layer with glial processes extending connections to the tectum. This facilitated lesioning the transplant without damaging the tectum. Degeneration analysis after these lesions showed projections only to the visual nuclei. The heaviest of these projections was to the superficial layers of the tectum, the stratum opticum and stratum griseum superciliaris. There were also projections to the red nucleus, dorsal terminations of the red nucleus and the dorsolateral reticular nucleus. The discrete efferent projections of retinal transplants contrast with the broad distribution of connections from cortex and tectum, and are consistent with the same specificity. (Supported by USPHS Grant EY-01950 from the NIH.)

CHRONIC INTRAVENTRICULAR ADMINISTRATION OF LSD AFFECTS THE SENSI­TIVITY OF CORTICAL CELLS TO MONOAMINE DEPRIVATION. Maureen A.
McCull, David G. Tieman*, and Helmut V.B. Hirsch. Neurochemistry
Research Center, State University of New York at Albany, Albany, NY 12222.

In the present study, we have studied the effects of intraventricular administration of LSD on the sensitivity of cortical
cells in adult cats. Cats were assigned randomly to one of four conditions: MD/LSD; MD/no-LSD; no-MD/LSD; no-MD/no-LSD. An
chamical mini-pump (Alza Corp) was placed subcutaneously, and at­
ached to a cannula made from a 25-gauge needle which was implant­
ed in one lateral ventricle. The pump delivered either LSD tarte­
rate (0.88 µg/kg/hr dissolved in normal saline at pH 5.5) or the
vehicle solution alone for a period of one week. In addition, at the time of implantation, the lids of one eye of the MD ani­
mals were incised and a transplant of embryonic retina was per­
fected. (Supported by USPHS Grant EY0186164 and by Alfred P. Sloan Foundation Fellowship 18167.)

The mechanisms which guide the paths of growing neurites are fundamental to the formation of specific neuronal connections and establishment of functional neural circuitry. We addressed the problem of neural pathfinding in the context of a regenerating system by taking advantage of a well-known feature of a mammalian molecular layer. Neurons that normally have homogeneously distributed receptors in the dentate gyrus can be reliably relocated within different individuals of a given species. We employed the small, Helisoma trivolvis, for these studies and have centered our studies around two pairs of large identified neurons in the buccal ganglia, neurons 4 (R&L) which normally have axons in the esophageal trunk (ET) and other two-thirds of the molecular layer. Also, the stratum lacunosum-moleculare contains the lowest amount of RCA binding in the hippocampal formation. This is the case for Con A binding as well.

The hippocampus was studied at 3 or 30 days after a unilateral entorhinal lesion. At 3 days post-lesion no changes were found in Con A binding. However, at 30 days post-lesion an alteration of these lectin receptors patterns was seen. The amount of Con A (100 µg/ml) binding increased greatly in the entire molecular layer but "avoids" the GN. Neuron 5 normally has a large axon in the buccal mass. Neuron 4 normally has an axon branch in the SN and occupies the entire stratum radiatum and stratum lacunosum-moleculare. In addition, there is a band of intensified staining at the interface between the first and second third of the molecular layer. Otherwise the reaction product appears homogeneous throughout the remainder of the molecular layer and stratum radiatum.

Ricinus communis agglutinin (RCA) (200 µg/ml) also shows homogeneous staining throughout the stratum radiatum and outer two-thirds of the molecular layer. Like Con A and FBP, there is intensified staining in the first third of the molecular layer. Also, the stratum lacunosum-moleculare contains the lowest amount of RCA binding in the hippocampal formation. This is the case for Con A binding as well.

Vocal control pathways of the canary brain include two discrete telencephalic nuclei, hyperstriatum ventrale, pars caudale (HVc) and nucleus robustus archistriatalis (RA). These two stations show gross sexual dimorphism, presumably related to the fact that males, but not females, sing complex, learned songs (Nottebohm and Arnold, Science 212: 763). These two stations are immediately adjacent to each other and are the only centers with similar anatomy that are affected by either gonadectomy or ovariectomy in both males and females. In male canaries, HVc and RA seem to be unique in the extent to which gross neural plasticity normally associated with early development can be induced in adulthood at a time when auditory-motor vocal experience may be integrated into HVc and RA patterns of growth and connectivity.

In a separate experiment, 10 male and 10 female canaries were gonadectomized 5-19 days after hatching. Half of the ovariec­tomized females received silastic estrogen (E) implants, the other half received silastic testosterone (T) implants at 11 months. Both groups of gonadectomized females, 6 intact females of the same age, the castrate males and 10 intact male siblings of the latter were sacrificed at 12 months of age. Attempts were made to record song from all these birds. All intact males deve­loped adult song. The castrate males produced songsubsonic and plastic songs, but in all cases at approximately 8 months, when their intact siblings were coming into stable adult song as well as into repro­ductive condition. Of the females, only the T-treated group pro­duced male-like song. HVc and RA volumes were reconstructed for all these birds. HVc and RA were 90% and 53% larger, respectiv­ely, in the T-treated than in the C-treated females. HVc and RA were 45% and 54% smaller in the castrate than in the intact males. The ratio of male/female HVc and RA was comparable in intact and castrate siblings.

The 30th to 60th day period of rapid growth coincides with the growth and connectivity.

The present study was conducted to confirm that recovery of alternation performance co-occurs with sprouting (Loesche and Steward, 1977). Thirty-six food-deprived male rats were trained to alternate for food reward in a Y-maze in which the recess of the right arm always contained a food reward. Animals were weighed, fixed in formalin, embedded in gelatin albumen, sectioned at 50 μ intervals and stained with cresyl violet. All age groups had adult brain volumes. The volume of HVc and RA was reconstructed as described in lit. HVc is first recognizable on day 30 after hatching. At this age RA and HVc volumes are 30% and 25%, respectively, as large as those of 1-year-old males in full reproductive condition. HVc and RA grew by a factor of 2.5 between the 30th and 60th post-hatching day. Adult volume was reached, respectively, by the end of the third and fourth month. The 30th to 60th day period of rapid growth coincides with the subsong stage of song development, a time when auditory-motor vocal experience may be integrated into HVc and RA patterns of growth and connectivity.

The results obtained for experimental and control animals were similar to those observed in degenerating axons. At earlier stages of degeneration, synapses retained their postsynaptic contacts, while at more advanced stages glial processes frequently engulfed the terminals. These results demonstrate that regrowing pyramidal tract fibers form synaptic terminals in their appropriate target areas. This suggests a mechanism for possible recovery of function. The results obtained for experimental and control animals were qualitatively similar. The fibers of both the normal and regrowing pyramidal tract showed similar rates of degeneration. After 8 days survival, myelinated axons exhibited various stages of degeneration. There were intact degenerating mitochondria and dense bodies, but their microtubules and microfilaments appeared normal. Other axons in a more advanced state of degeneration were completely filled with a dense granular matrix. Degenerating axons were similar in appearance but far less numerous at 2 days than at 9 days.

Electron microscopic evidence for establishment of synaptic connections by regrowing pyramidal tract axons in the infant hamster. T. Reh and K. Kaali. Neurosciences Training Program and Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

In a previous report (Kaali and Reh, '78) we presented light microscopic autoradiographic evidence demonstrating that pyramidal tract axons severed in the medulla of infant hamsters are capable of regrowing to long distances to innervate their normal targets in the medulla and spinal cord, albeit by an abnormal route. We now present electron microscopic evidence that the regrowing fibers form synapses in at least two areas of these terminal regions, the dorsal column nuclei (DCN) and the dorsal horn of the cervical spinal cord.

Electron micrograph from a rat 63 days after a 4-4.5 day lesion of the pyramidal tract at the level of the inferior olive and allowed to survive to adulthood. The sensorimotor cortex ipsilateral to the lesion was then removed and the animals sacrificed 2-8 days after the cortical lesion. The tissue was processed for electron microscopy and thin sections through the DCN and cervical spinal cord examined for degenerating axons and terminals. Sections taken from identical areas of normal animals with similar cortical lesions served as control material. The results obtained for experimental and control animals were qualitatively similar. The fibers of both the normal and regrowing pyramidal tract showed similar rates of degeneration. After 8 days survival, myelinated axons exhibited various stages of degeneration. There were intact degenerating mitochondria and dense bodies, but their microtubules and microfilaments appeared normal. Other axons in a more advanced state of degeneration were completely filled with a dense granular matrix. Degenerating axons were similar in appearance but far less numerous at 2 days than at 9 days.

Synaptic terminals in the DCN and cervical spinal cord contain an accumulation of dense bodies and darkened cytoplasm similar to that observed in degenerating axons. At earlier stages of degeneration, the synapses retained their postsynaptic contacts, while at more advanced stages glial processes frequently engulfed the terminals. These results demonstrate that regrowing pyramidal tract fibers form synaptic terminals in their appropriate target areas. This suggests a mechanism for possible recovery of function. (Supported by NIMH grant HD-14428 and NIMH training grant MH 07507.)

Multiple end plate potentials (e.p.p.s.) can be recorded in single muscle fibers innervated by several motor neurons when the whole motor nerve is stimulated by successive impulses of increasing intensity. Such multiple e.p.p.s. were recorded in 16% of the fibers comprising cutaneous-pectoral muscles of normal frogs. The number of muscle fibers in which multiple e.p.p.s. were recorded increased in intact right muscles after injuring the motor nerve to the left muscle. In this respect, the incidence of polymuneuronal innervation attained by the formation of new synapses and if so where do new axon terminals arise. From where does the new sprouted neuron branch and how do they grow upon the muscle fiber they innervate? In contrast, in intact right muscles of experimental frogs the incidence of polymuneuronal innervation was significantly greater (0.001 level) for the experimental population (range: 19% to -27%; SD = ± 10.7) than for the controls (range: 11% to 10%) with muscle fibers apparently not innervated by their parent axons. The number of axons comprising the motor nerve to right intact muscles and left denervated muscles was similar (counts made proximal to site of injury). In normal frogs, muscle fibers comprising cutaneous-pectoral muscles are innervated at a single end plate region that can be marked histologically. The mechanism controlling this site of innervation is unknown which is confined to the synaptic site only. The average end plate size in right muscles is within ± 10% of that of left muscles. However, there was a decrease in the average end plate size in right intact muscles over that in left muscles. In the same right muscles there was an increase in the average end plate size in right intact muscles over that in left muscles. A decrease in the average end plate size in right intact muscles over that in left muscles. A decrease in the average end plate size in right intact muscles over that in left muscles. A decrease in the average end plate size in right intact muscles over that in left muscles.
Potentially missing: 2166, 2167, 2168

2164


In alpha-bungarotoxin (5nM) was found to be effective in blocking the synapses of all three classes of retinal fibers in goldfish tectum. It severely decreasements the tectal potentials on the retina as well as mediated projections of tectum. The current contour-density further shows that it blocks the salient and the peaks synchronous to maximal activation which is related to lateral inhibition. In some cases, the lateral inhibition is slightly different when the lateral inhibition was recorded. In all cases, the lateral inhibition is less than the lateral inhibition recorded by the left eye and there was an abrupt transition to units activated only by the right eye as more lateral regions were sampled. Whether the lateral inhibition was recorded in the left or right eye was also dependent on the side of the lesion.

2165

DEVELOPMENTAL ALTERATIONS IN BINOCULAR COMPETITION AND VISUAL ACUITY IN VISUALLY-DEPRIVED CATS. Douglas C. Smith, Harris D. Spear, 1976). This research was supported in part by a NIMH Grant #MH 17345 to A.J.N., a Sigma Xi Grant-in-Aid to C.L.S. and the University of Kentucky Graduate School.

In this study, we investigated the effects of monocular deprivation on the development of binocular organization and visual acuity in kittens. We found that kittens deprived of vision in one eye showed severe deficits in visual acuity, while kittens with normal vision in both eyes showed normal or near-normal acuity. The kittens were divided into two groups: one group was deprived of vision in the right eye, and the other group was deprived of vision in the left eye. The kittens were tested at various ages to determine the effects of deprivation on their visual acuity.

2166


In an attempt to distinguish between sparing and recovery of function within the 'prefrontal system' of the rat, 7-8 day-old infant rat pups were given medial frontal (MF), orbital frontal (OF), caudate nucleus (CN) and sham (SH) lesions allowed to recover and then tested at 14-days of age on a variety of tasks known to be sensitive to prefrontal damage in the adult rat. These tasks include resistance to extinction of a learned approach response, passive avoidance, spatial discrimination and visual discrimination in the open-field. They were also tested on an odor-avoidance task. Additional rat pups 10-14 days old without lesion were tested on the same tasks to determine if there were any age-dependent differences in performance within this age range which might interact with the lesion-dependent changes in performance. The results indicated that MF lesions produced no deficits on any of the tasks employed. In addition, only the CN operated showed an increased resistance to extinction and an increased number of trials to passive avoidance as compared to SH pups. Not all MF and OF lesions, however, had resulted in spatial reversal and demonstrated an increase in activity as compared to sham. All lesioned and SH pups showed a decrease in activity as compared to normals.

2167

ELECTROPHYSIOLOGICAL MAPPING OF aberrant RETINAL PROJECTIONS TO THE SAME SUPERIOR COLLICULUS IN HAMSTERS. George M. Sachs* and Gerald E. Schneider. Department of Psychology, N.I.T., Cambridge, MA 02139.

After ablation of the superficial layers of the right superior colliculus (SC) in newborn hamsters, retinal fibers from the left retina recross the midline at the tectum and terminate in a medial portion of the intact left SC. Neuroanatomical studies have shown that these aberrant projections are from the normal contralateral retinotectal projection. Twelve weeks to one year after early right tectum ablation, 12 hamsters underwent acute removal of visual cortex, and visually evoked multi-unit and single-unit responses were recorded from the left SC. Five hamsters also received injections of H-uracil in the left eye 4 days before recording.

Electrophysiological results indicated that the tectal projections from the two eyes exhibited varying degrees of overlap. For some cases, tectal units recorded medially were driven exclusively by the left eye and there was an abrupt transition to units activated only by the right eye as more lateral regions were sampled. In cases where the aberrant projection appeared less dense as seen with autoradiography, there was electrophysiological evidence for overlap of the two retinal projections. From some sites two groups of units, each driven by a different eye, could be recorded simultaneously. These sites were encountered in areas in the two retinae were represented in two different topographic maps within the same SC.

Supported primarily by NIH grant EYO0126.
PLASTICITY


Previously, we have described "periependymal" cell proliferation in the goldfish optic tectum. Labeling among these cells, whose nuclei are located between the ependymal and the neuronal stratum periventriculare (SPV), is seen when 3H-thymidine injections are made 25-35 days after optic nerve section, neuronal stratum periventriculare (SPV), is seen when 3H-thy­

mide injections are made 25-35 days after optic nerve section, the time at which functional reinnervation of the tectum occurs. Tecta denervated by eye enucleation do not show such prolifer­

ation (Stevenson and Yoon, '78, Brain Res., 153).

The present report describes the periependymal (PE) cells. In the light microscope these cells are easily recognized by their location and thymidine labeling properties. Unlabeled PE cells are distinguished from their neighbors by their deeply invag­

inated nuclei with eccentric nucleoli, moderately basophilic cytoplasm and large perikarya. Golgi preparations display a single peripherally directed process extending from such per­i­

karya. The process reaches the stratum opticum where it divides into several oblique branches which terminate at the tectal surface as subpial end feet. Along its course the process is covered in myelin sheaths, giving it the appearance of a spruce tree. Neither the Golgi rapid nor the Kopetz technique demon­

strates an apical process connecting the PE cell perikaryon with the ventricle. PE cells contain its invaginated nucleus, abundant Golgi apparatus, mitochondria which often contain single dark granules, rough and smooth endoplasmic reticulum, free ribosomes, and a great number of microtubules which funnel into the process where they run longitudinally.

The notable absence of fine filaments and an apical process connecting the perikaryon with the ventricle distinguish the PE cells from other radial glia in the goldfish tectum.


Following the removal of the major excitatory projection to the first dentate nucleus, the ipsilateral entorhinal cortex, the dentate granule cells are reinnervated as a result of the sprouting of several surviving afferent systems. The present study examines uptake of 2DG in the dentate gyrus to determine, a) if the removal of a major excita­

tory pathway would decrease 2DG uptake in the denervated dentate ganglion, and b) whether any decreases would be reversed as a consequence of the reinnervation. 2DG uptake was measured auto­

radiographically at 1,2,4,8,10, and 14 days post-lesion by in­

jecting the animals with 2DG 45 min. before sacrifice, and processing the tissue on emulsion-coated slides, permitting a quantitative evaluation of 2DG uptake through grain counting. For comparison, a few animals were also injected with 14C 2DG, and processed on x-ray film. 2DG uptake in the denerv­

ated dentate gyrus ipsilateral to the lesion was compared with that in a comparable region on the contralateral side. The 2DG uptake was reduced in the denervated dentate gyrus during the early post-lesion intervals (1,2, and 4 days), although the ex­

tent of the decrease is surprisingly slight (approximately 20%) given the density of the entorhinal projection system. At 8-10 days post-lesion, 2DG uptake in the denervated zone was not only not decreased, but was actually somewhat increased relative to the contralateral control. This increased uptake was restrict­

ed to the outer portion of the dentate stratum molecular. The enhanced uptake was not evident at the 14 days post-lesion. These results suggest that denervation results in a reinnervation of the 2DG uptake, while reinnervation (which occurs predominantly around 8-­

10 days post-lesion) is accompanied by a reversal of these de­

creases, and even a slight increase in the relative contralateral control during the period of active symptomatology.

The question of whether this increase at 8-10 days post-lesion reflects a metabolic accompaniment of the active phase of the reinnervation, or simply a return of excitatory innervation will re­

quire a further analysis at longer post-lesion intervals, after the lesion-induced symptomatology has reached completion. Be­

cause the extent of the changes was so slight given the density of the entorhinal projection system, it is probable that most of the 2DG uptake in areas of neuropil in the hippocampus is asso­

ciated with elements other than afferents and terminals. The re­

sults demonstrate the usefulness of the 3H-2DG method for quanti­

tative studies.

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Comparison of the brains of rats raised for a month or more in a stimulating environment with those of siblings raised in a more repressing environment will facilitate the study of the differences in neural development. In this way a variety of components of the neural microenvironment have been found to be susceptible to the dif­

ferences which appear to exist between the two environments. These differences in the thickness of the myelinated pathway at the base of the occipital cortex, above the corpus callosum. This size difference was apparently the result of greater numbers of axons in enriched cortex. The general appearance of the myelinated pathway is asso­

ciated with elements other than afferents and terminals. The path­

ways for which there presently are adequate samples include the cingulum, the fornix and the stria terminalis. These pathways is by perfusion with mixed aldehydes and fixation in osmium and was then embedded in Epon. Semi thin sections of the tissue were stained with toluidine blue. There is data available for the dorsal root afferents are the major known source of SP in the spinal cord. Using the unlabeled antibody (PAP) technique for demonstrating SP, we have shown that following lumbarosacral de­

afferentation of the spinal cord, SP reaction product in the dorsal horns of lumbar segments progressively decreases for the first 10-11 days after surgery. However, by 8-10 days post-lesion, a significant change in the dorsal horns of the lumbar segments rostral to the transection. This suggests that deafferentation combined with mid lumbar (L4) transection did not prevent the return of SP reaction product to the dorsal horn of segments rostral to the transection. This suggests that ascend­

ing collaterals may be involved in the reinnervation process. In fact, SP containing cells are seen in the lumbar grey matter in normal and operated animals. These cells are located in the lateral and intermediate laminae, VII, VIII, and X, of lumbar segments both on the side of deafferentation and on the side with intact dorsal roots. The SP containing cell bodies do not constitute a specific nucleus but are scattered among neurones in the lateral and intermediate laminae. Moreover, the fact that they are also dispersed rostrocaudally while the return of SP is found in all deafferented lumbar segments, suggests that the SP cells main­

tain widespread projection systems which extend rostrocaudally for several segments. Sprouting by interneurons may provide one explanation for the hyperreflexia which occurs in chronic, de­

afferented animals.


The source of the SP which returns after deafferentation remains unknown. Deafferentation combined with transection at the L1 level did not prevent SP reaction product from returning amounts comparable to those seen after 1 month survival with de­

afferentation alone. These data suggest that long descending tracts are not the sources of the SP which returns after deafferentation. Deafferentation combined with lumbar (L4) transection did not prevent the return of SP reaction product to the dorsal horn of segments rostral to the transection. This suggests that ascend­

ing collaterals may be involved in the reinnervation process. In fact, SP containing cell bodies are seen in the lumbar grey matter in normal and operated animals. These cells are located in the lateral and intermediate laminae, VII, VIII, and X, of lumbar segments both on the side of deafferentation and on the side with intact dorsal roots. The SP containing cell bodies do not constitute a specific nucleus but are scattered among neurones in the lateral and intermediate laminae. Moreover, the fact that they are also dispersed rostrocaudally while the return of SP is found in all deafferented lumbar segments, suggests that the SP cells main­

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afferented animals.

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EVIDENCE FOR THE INVOLVEMENT OF THE RETROGRADE REACTION IN SUSTAINED COLLATERALS DURING RECOVERY OF FUNCTION FOLLOWING BRAIN DAMAGE. Roy G. Thompson* and Fred H. Gage. Chemistry of Behavior Program, Texas Christian University, Fort Worth, Tx 76129.

Present understanding of neural mechanisms subserving recovery of function following brain damage is severely impaired by various means of compensating for the loss of neural input, e.g., collateral sprouting and receptor supersensitivity. Reversible consequences of neural damage are currently understudied. Recent lines of evidence have recently demonstrated that nerve terminals proximal to the site of damage exhibit a transient decrease in both their neuro transmitter content and biosynthetic enzymes. These data have been interpreted to reflect the occurrence of a functional de nervation at the terminal sites of axon collaterals which remain structurally intact following axotomy. The purpose of the pre sent experiment was to further investigate the consequences of axotomy on the functional integrity of sustained collaterals. The ability of hypothalamic nerve terminals to accumulate and retain their neurotransmitter was determined at various times following septal lesions and compared with changes in uptake occurring in nerve terminals exhibiting anterograde degeneration. Crude synaptosomes were prepared from hippocampal and hypothalamic tissue at 1, 5, 10 and 15 days following septal lesions and incubated in the presence of triitated noradrenaline, serotonin or choline. The results show that uptake of all three transmitter substances is decreased by 20-30% on the first day after septal lesions in both hippocampus and hypothalamus. Maximal loss of uptake was observed by day 5. While loss of hippocampal uptake remains depressed at 10 and 15 day survival times, hypoth alamic uptake returns to normal by day 15. The initial decrease in uptake observed in the hypothalamus lends support to the hypothesis of a disruption in the functional integrity of proximal nerve terminals following axotomy. Recovery of hypothalamic uptake can be interpreted to reflect one of two events: 1) collateral sprouting of intact axons in response to depen dergy septo-hypothalamic projections, or 2) recovery of uptake within nerve terminals exhibiting the initial loss. A kinetic analysis of neurotransmitter uptake is currently being performed in order to further elucidate the neural events contributing to this recovery.


Long-term potentiation (LTP) in the hippocampus has been the subject of considerable interest in recent years as it represents a potential model of learning and memory. LTP is routinely produced in the hippocampal slice preparation by a brief period of electrical stimulation of the Schaffer collaterals and commissural fibers. However, the biochemical processes underlying this long-term change in synaptic efficacy are not yet understood.

The present study examined the possibility that LTP in the hippocampal slices is accompanied by changes in the accumulation of the excitatory amino acids glutamate and aspartate, which are possible neurotransmitters in the potentiated pathways. PG ppted slices incubated with a low (50 nm) concentration of \( ^{3}H \)-glutamate accumulated more radioactivity (40%) than did control non-stimulated slices. Further, this increase was still present 30 minutes after the stimulation. A similar increase in accumulation of radioactivity was exhibited in potentiated slices incubated with \( ^{3}C \)- or \( ^{14}C \)-aspartate. It is not likely that the increased accumulation found with the excitatory amino acids represents a nonspecific effect since potentiated slices incubated with \( ^{14}C \)-tyrosine or \( ^{14}C \)-glucose showed no increase in the accumulation of radioactivity. In addition, the increased accumulation of \( ^{3}H \)-glutamate was more pronounced in the regio more, the lower portion of the dentate gyrus, and terminals, than in the dentate gyrus. Under the present conditions, the accumulation of \( ^{3}H \)-glutamate by slices is not likely to simply reflect uptake by presynaptic elements since, under several lines of evidence accumulation by slices is not changed whereas the uptake in homogenate is decreased by 30%. Thus, the present results are consistent with the hypothesis that LTP induces increased number of binding sites for glutamate. (Supported by NIH grant 17979 and NSF grant 7027137).

AGE-RELATED COMMISSURAL SPROUTING IN THE DENTATE GYRUS DEMONSTRATED BY ANTEROGRADE TRANSPORT OF HORSERADISH Peroxidase. James R. West, Aes C. Black, Jr., and Terence H. Williams. Department of Anatomy, University of Iowa, College of Medicine, Iowa City, Iowa 52242.

Using a modification of the horseradish peroxidase technique (West and Black, Neuroscience Letters, in press), we studied post-lesion growth in the dentate gyrus. In accordance with Lynch and co-workers, we found moderate growth of the commissuralterminal band into the outer molecular layer in rats receiving unilateral entorhinal lesions as adults (group A). In rats lesioned at 11 days of age and examined as adults (group B), we observed prolific growth throughout all but the most distal edge of the outer molecular layer.

The density and distribution of label in group A suggests that the sprouting probably results from direct expansion of fibers and terminals into the proximal portion of the lesion-induced deafferented zone. In group B, particularly in the dorsal leaf of the dentate gyrus, the label appeared as two dense bands (the proximal band corresponded to the normal commissural terminal field), separated by an area of lighter labeling. This disparity in patterns of re-innervation between groups A and B suggests that the mechanisms underlying the reorganization might not be the same.

We suggest that one of the following mechanisms is responsible for these results: (1) The commissural fibers grow into the normal zone and then sprout into the outer molecular layer. Their terminals are distributed to two different portions of the den­ritic tree, creating the appearance of two separate bands. (2) There is a division of the commissural fibers as they grow into the altered dentate gyrus during development, creating two adjacent bands. (3) A combination of the above mechanisms is also possible, e.g., two bands result from the division of the commissural fibers as they enter the dentate gyrus. The inner fiber plexus could then sprout; the sprouts occupying the space between the two bands. (Supported by NS 11650 to THW).


We have assessed the effects of auditory deprivation in the cortex of the mongolian gerbil. Compared to littermate controls 32 day old experimental animals showed significantly lower spine densities along pyramidal cell apical dendrites. Ligation of the external meatuses at day 12 caused acoustic deprivation of more than 25 decibels. Groups of gerbils with sacrificed 12, 22 and 32 days after birth and cerebral blocks were processed for rapid Golgi. The auditory cortex was mapped in a parallel electrophysiological study and all potential experimental criteria. Electrolytic lesions in Nissl stained material pro­vided a reference for Golgi quantification. This quantification was restricted to pyramidal cells of layer V in primary auditory cortex. Drawings of all well impregnated neurons were made at a magnification of 1000 X using a drawing tube and spine densities were determined from these drawings. 50 µm long segments of the apical shaft beginning 150 µm above the soma was quantified. In 32 day old deprived material spine densities were lower an average of 38% in experiments (p < .005; n of cells: 14 control, 15 experimental). A comparison of the entire 200 µm segment also yielded significantly lower densities in experiments (p < .005). An insufficient amount of 22 day old control material precluded a similar analysis at this age. However, a comparison between the 22 and 32 day day old deprived groups showed signifi­cantly higher densities in the younger material. Additionally there is no significant difference between 22 day old deprived and 32 day old control material (mean per 10 µm is 12.1 and 10.7 spines respectively). These data suggest that the lower spine density seen in the month old gerbils is due to a reduction from a state of higher spine density rather than being the result of delayed maturation.

Supported in part by PHS grant AG01018-01 and U.C.D. Chancellor's Patent Fund U381.

Cytochrome oxidase histochemistry has been applied previously in the study of functional changes within the auditory and visual systems of cats (Wong-Riley, Merzenich & Leake, Brain Res. 141: 185-192, 1978; and Wong-Riley, Brain Res., in press). The present study examines the applicability of this technique in the somatosensory system of rodents. The barrels in layer IV of normal mouse SmI face cortex were found to have a high level of cytochrome oxidase activity. Surrounding each barrel was a clear zone of very low enzyme reaction. Preliminary electron microscopic examination indicated that most of the reactive mitochondria within the barrels reside in the neuropil, rather than in the cell bodies. Thus, the barrels normally have a higher level of oxidative metabolism, most likely reflecting a higher level of functional activity, than surrounding regions. In order to determine whether vibrissal damage induces any change in the oxidative activity of the neonate and adult animals, and whether the C.O. technique is sensitive enough to detect such functional changes in the somatosensory cortex, two groups of mice were tested. In the first group, selective row or rows of mystacial vibrissae were cauterized on postnatal day 1 (within hours after birth), and the animals allowed to survive for 2-3 months. In the second group, vibrissal cauterization was performed in adult animals, and they survived for 2-3 months post-operatively. The results indicated that neonatal removal of vibrissae caused severe shrinkage and often fusion of affected cortical barrels, with concomitant expansion of neighboring barrels (confirming previous reports by others), and the level of cytochrome oxidase activity of the shrunken barrels was lower than that of normal. Removal of vibrissae in the adult did not cause size changes of cortical barrels. However, there is a significant decrease in the level of oxidative enzymatic activity within these barrels. Thus, the removal of sensory input through the destruction of peripheral sensory organ causes distinct functional changes at the level of the cortex. Such changes are more severe in the neonate, where cortical fields actually shrink in size. Changes, however, can still be induced in the adult animal, where the levels of oxidative activity of cortical fields are demonstrably reduced. These functional changes are detectable morphologically with the cytochrome oxidase technique.
PSYCHO-PHARMACOLOGY
EFFECTS OF APOMORPHINE ON ELICITED AND OPERANT PECKING IN PIGEONS.

Joanne S. AbeIson* (SPON: B.P.H. Poschel). Psychology Department, but some subjects show paradoxical rate-increases. To account for such findings, it has been postulated that an occasional subject might redirect its stereotyped responding toward the operant key, and thereby generate high operant rates. The relationship between the effects of APO on elicited (stereotyped) and operant behavior was studied by examining the effects of the drug on both behaviors in the same subject.

Fifteen test- and drug-sophisticated pigeons were used as subjects. In both experiments, the pigeons were injected i.p. with 3.2, 3.6 or 4.0 g/kg ethanol (20% w/v). The effects of APO were determined by comparing the rate of pecking on the operant key with the rate of pecking on a multiple FI-5, FR-30 schedule revealed individual differences in response to the drug. These differences were used to form descriptive groups based on the lowest dose which would eliminate operant responding. Doses of 0.32 mg/kg eliminated all operant responding in four subjects (Group 1); 3.2 mg/kg was needed in some other subjects (Group 2); and two subjects required 32.0 mg/kg to eliminate responding (Group 3). Groups 1 and 2 showed similarities in overall drug effects and differed only in the sensitivity of their stereotypy to the action of APO. The highest group showed dose-related decreases in operant responding with concomitant increases in elicited behavior. In contrast, data from group 3 showed increases in operant responding which were highly correlated with increases in stereotypy.

It is concluded that the effects of APO on operant behavior were dependent on induced stereotypy. Rate-decreasing effects appear to be related to the disruption of ongoing operant behavior by elicited pecking aimed elsewhere in the chamber, while rate-increases seemed to be produced by the redirection of elicited pecking to the operant key. Further, since the operant behavior showed similar individual differences in sensitivity to APO while elicited behavior did not, operant and elicited pecking may be differentially mediated by dopaminergic mechanisms. This separation of the key-peck response into two components may provide a model for the screening of neuroleptic drugs.

PHYSIOPSYCH Pharmacology

EFFECT OF STRAIN DIFFERENCES IN TYROSINE HYDROXYLASE ON PHENCYCLIDINE-INDUCED LOCOMOTOR ACTIVITY

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Phencyclidine-induced locomotor activity was studied in three strains of albino rat that differ in the level of tyrosine hydroxylase (TH) activity in the brain (Segal, et al., 1972). The strains included: F344 (high in TH), Sprague-Dawley (moderate level of TH), and BuF (low in TH levels). Activity level following 0.5-4.0 mg/kg phencyclidine (PCP) was recorded for a 30 minute period in a circular horizontal runway with each animal receiving a baseline session 24 hours prior to drug treatment. Activity was measured for each strain received saline on the day of PCP treatment. There was a significant dose-response curve for each strain. Activity increased in a linear way with increasing dose level. Further, there were significant differences between the strains in the amount of activity induced by PCP. Because of these differences, a transformation of the data was made and the treatment effects analyzed through analysis of variance methods. The F344 strain was significantly more sensitive to PCP at 2.0 and 4.0 mg/kg. The BuF strain was the least sensitive to the effects of PCP.

These results when interpreted in view of the TOH differences across these strains and the response of these strains to norepinephrine and amphetamine, suggest that PCP does act like a sympathomimetic. It is consistent with this suggestion that strain differences in catecholamine receptor activity associated with the TOH differences would suggest that the mechanism in responsiveness to PCP as measured in the present study.

ONTOGEDY OF STRAIN DIFFERENCES IN NIGROSTRIATAL THIOSE HYDROXYLASE ACTIVITY AND SPONTANEOUS AND DRUG-INDUCED BEHAVIOR IN MICE.


Adult mice of the BALB/cJ strain have more dopamine (DA) neurons and greater tyrosine hydroxylase (TH) activity in the substantia nigra-pigmentosus (SN) region, caudate nucleus (CN), and mesolimbic brain areas than mice of the C57/B1J strain (Nature 264:964, 1976). These differences correlate with greater spontaneous and amphetamine-induced locomotion and lesser sensitivity to apomorphine stereotypy in BALB/cJ mice (Neurosci. Abst. 4:492, 1978). We sought to determine: (a) if the strain-dependent differences in TH activity in SN and a major terminal field, the CN, develop postnatally; and, (b) whether there is a temporal correlation between the ontogeny of the differences in TH and the behavioral differences in these two strains. In both strains, TH activity in SN increased rapidly, peaked at 125% of adult levels 15 days postnatally (P15) and decreased gradually to adult levels (P56) at P12. TH in the CN increased gradually, with TH activity at 65% of adult levels at P15 and 90% of adult levels at P21. Strain differences in TH activity (BALB/cJ > C57/B1J) were not present before P7 but, first appeared in the SN at P9 and in the CN at P11. Choline acetyltransferase (Chat), a marker for the integrity of intrinsic neurons of the CN, was 40% of adult levels at P15 and 70% of adult levels at P21. In early development (P8-P15), spontaneous and amphetamine-induced locomotion were less than, and apomorphine stereotypy greater than or equal to, the adult values. At this time, in contrast to adults, BALB/cJ mice were less active, less responsive to amphetamine, and more responsive to apomorphine than C57/B1J mice. Between P15 and P21 spontaneous and amphetamine-induced locomotion increased markedly in both strains. Our results suggest that apomorphine stereotypy decreases in BALB/cJ mice and increased in C57/B1J mice. By P21, mice evident strain differences in spontaneous and amphetamine-induced behaviors which paralleled the adult pattern. This corroboration suggests: (a) strain differences in TH activity in the nigrostriatal system and the adult pattern of DA-mediated behaviors first appear postnatally; and, (b) that the development of the differences in TH activity in the nigrostriatal system precede the adult patterns of strain differences in DA-mediated behaviors. These data suggest that the phenotypic expression of TOH-related behaviors is dependent not only on the level of TH activity in the nigrostriatal system, but may also depend on the maturation of its major afferent nucleus, the SN.
BLOOD PLASMA OSMOLALITY AND MANIC-DEPRESSIVE ILLNESS


Rhesus monkeys were trained to discriminate between left- and right-opening Landolt rings. The test apparatus presented stimuli at 1 meter in seven discrete sizes corresponding to Snellen acuities 20/200, 20/100, 20/50, 20/40, 20/30, 20/20 and 20/15; a titration paradigm was designed to elicit performance at maximum acuity. Upon establishment of stable baseline performance, the subjects were injected IM with the following drugs prior to testing: (1) benactyzine, 0.054 to 0.54 mg/kg; (2) atropine, 0.014 to 0.14 mg/kg; (3) TAB (a compound consisting of atropine, benactyzine, and the oxime TMB-4) in a ratio of 1:4:40, 0.64 to 6.4 mg/kg; and (4) a placebo control. Injection of active substances caused dose-related decreases of near visual acuity. Onset times and effect durations differed among drugs.

EFFECTS OF ANTICHOLINERGICS ON VISUAL ACUITY OF MACACA MULATTA. C. T. Bennett, G. D. Callin, D. N. Farrer, C. Link and F. Garcia (SPON: James King). USUH School of Aerospace Medicine/R2M, Brooks AFB, TX 78235.

There is evidence to suggest that enkephalins may modulate the normal functioning of the nigro-striatal dopamine system by interacting with opiate binding sites in the brain. Given this and the recent finding that chronic naloxone administration produces an increase in opiate receptor binding sites, we undertook to investigate the effect of chronic naloxone pretreatment on the effects produced by the dopamine agonists, d-amphetamine and apomorphine.

Thirty male Wistar rats received a daily injection (subcutaneous) of saline (1ml/kg) or naloxone (5mg/kg) for eight consecutive days. Forty-eight hours after the last naloxone injection, animals received an intraperitoneal injection (1.7 F) of either d-amphetamine (3 mg/kg), apomorphine (3mg/kg) or saline (1ml/kg). Fifteen minutes after the 1.7 F injection, the animal's locomotor activity in an open field was measured by the number of photo-beams broken during the 30 minute testing session. The animals were restested seven and 14 days after the termination of the naloxone pretreatment using the same experimental procedure.

An analysis of variance revealed a significant overall increase in the activity by naloxone. Furthermore, a significant drug effect, as well as a significant overall decrease in activity following the naloxone pretreatment sessions were noted. Multiple t-tests revealed that amphetamine produced a greater activity in animals given naloxone pretreatment than in animals that received saline pretreatment.

The increase in locomotor activity observed in rats chronically treated with naloxone, as well as the marked potentiation of d-amphetamine's excitatory effect, suggests that prolonged blockade of the opiate receptor may lead to the development of a supersensitivity in the dopamine systems that mediates motor function. Based on this hypothesis, endogenous enkephalins excite striatal dopamine neurons, then an increase in the number of opiate binding sites produced by chronically administered potentiate the excitability of the nigro-striatal dopamine system.

Stereotyped behavior in animals is believed to result from stimulation of striatal dopaminergic mechanisms, however, it is now widely held that this behavior can be modulated by limbic dopaminergic and noradrenergic influences. Stereotyped behavior in rodents elicited by d-amphetamine (DA) has been used as a pharmacological paradigm for schizophrenia because this behavior is blocked by neuroleptics, and amphetamine administration in man can result in a paranoid schizophreniform psychosis. We have shown that the administration of phenylethylamine (PEA), an endogenous biogenic amine, is also capable of producing stereotypy. Dopamine receptor blocking antipsychotics antagonize both DA and PEA elicited stereotypy, however, neither DA nor PEA produces stereotypy in animals treated with these drugs. Moreover, the striatal administration of DA (100µg) resulted in a stereotypy score of 3.50 ± 0.11 with increased locomotion, and stereotyped grooming, sniffing and head-swinging. In contrast, intralimbic administration of DA (100µg) produced stereotyped sniffing, achieving a score of 2.62 ± 0.13. In contrast, intralimbic injection of PEA (100µg) resulted in a stereotypy score of 3.50 ± 0.11 with increased locomotion, and stereotyped grooming, sniffing and head-swinging. These results demonstrate that PEA stereotypy may more specifically involve limbic mechanisms. To test this hypothesis we have studied the effects of intrastriatal and intralimbic injections of dopamine, DA and PEA. Subjects were white male Sprague-Dawley rats with bilateral stereotactically placed 0.8mm stainless steel cannula in the caudaleputamen nucleus and the nucleus accumbens. Animals were rated for stereotypy using a 5 point scale of ascending intensity of stereotypy. The administration of dopamine (10µg) into the striatum produced 0.6mg/kg, sniffing and occasional gnawing resulting in a stereotypy score of 3.37 ± 0.12. In comparison, intrastriatal DA (100µg) elicited a stereotypy score of 4.25 ± 0.16 and increased locomotor activity. In contrast, DA (100µg) administration resulted in a score of 2.00 ± 0.01 with animals exhibiting stereotyped grooming and sniffing lasting 20 min. We found the administration increased locomotion, occasional gnawing, stereotyped grooming and sniffing. At a dose of dopamine of 100µg, the stereotypy score was 2.8 ± 0.12, whereas at 200µg the score reached 3.5 ± 0.14. The administration of DA (100µg) produced stereotyped sniffing, achieving a score of 2.62 ± 0.13. In contrast, intralimbic injection of DA (100µg) resulted in a stereotypy score of 3.50 ± 0.11 with increased locomotion, and stereotyped grooming, sniffing and head-swinging. These results demonstrate that PEA, as compared with DA, exerts more potent effects on stereotyped behavior via the activation of mesolimbic mechanisms.

AN AMPHETAMINE-INDUCED DYSKINESIA IN MONKEYS MEDIATED BY PERIPHERAL SYMPATHETIC STIMULATION. P. J. Bushnell* and C. M. Baysinger* (SPH: W. T. McKinney). University of Wisconsin Primate Laboratory, Madison, Wisconsin 53706.

A highly characteristic behavioral response to d-amphetamine injections (0.3-3.0 mg/kg, SC) in infant rhesus monkeys was identified. This response consisted of bizarre, catactonic-like postures and dyslinesias in which the animals' hands and/or feet appeared to be floating in mid-air. The term "floating limb" developed over the first 1-10 biweekly injections in monkeys tested beginning at 4 weeks of age, and over the first 2-4 semimonthly injections in monkeys of any age. When fully developed, the response to drug occurred with an average frequency of 7/min., and occupied on the average 30% of the animals' total ongoing behavior. While floating limb was most dramatically induced by d-amphetamine, peripherally-acting sympathomimetics including hydroxyamphetamine (0.6 mg/kg) and ephedrine (25 mg/kg) also produced qualitatively similar response. The increased intensity of the dyskinesia following d-amphetamine may have resulted from its central, attention-focusing action, and to its stimulation of the peripheral sympathetic nervous system. The peripheral mechanisms mediating floating limb are presently being explored with sympathomimetic drugs having specific alpha- and beta-receptor activities.

Since hydroxyamphetamine and other sympathomimetics lacking CNS activity have rarely been used as control substances in psychopharmacological research with amphetamine, these results call into question the assumption that the behavioral effects of amphetamine are mediated solely by its activity in the CNS.

AN IMMOLIZING STIMULUS SIGNIFICANTLY ATTENUATES BOTH AMPHETAMINE AND APOMORPHINE-INDUCED STEREOTYPES. A.R. Caggia and L.R. Chiodo. Psychobiology Program, Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have recently suggested that the behavioral effects of activating and immobilizing stimuli depend, at least in part, on their reciprocal influence on the functioning of brain dopamine (DA)-containing systems. For example, an activating stimulus, such as tail pressure (TP), can induce a variety of appetitive behaviors and the behavioral effects of CP are blocked by pharmacological blockade of brain DA activity. Moreover, the same TP procedure dramatically alters the firing rate of DA neurons in the substantia nigra of anesthetized rats. Conversely, an immobilizing stimulus, such as cervical pressure (CP), can antagonize both the behavioral and electrophysiological effects of TP. Consistent with our view that CP can block the behavioral effects of DA activation, we now show that CP drastically attenuates the stereotyped responses produced by DA agonists, amphetamine and apomorphine.

Overanesthetized female rats received 50 mg/kg (s.c.) of estradiol benzoate 48 and 24 hours prior to testing. The test consisted of three 30 sec. bouts of CP which were preceded and separated by one minute baseline observation periods. The tests were conducted 30 and 120 minutes after amphetamine and 30 minutes after apomorphine. CP, which was administered with a saline lubricated glass rod (3 mm diameter) inserted into the vagina and pressed firmly against the cervix, attenuated all aspects of stereotypy for 6, 12 and 18 mg/kg of amphetamine and 3 and 6 mg/kg of apomorphine. For example, 60 minutes after 12 mg/kg of amphetamine, 85% of the animals were sniffing and 70% licking prior to CP, whereas percentages were 15 and 18%, respectively, during and after CP. Similarly, the values for 6 mg/kg of apomorphine were 100% sniffing prior to CP, 3% during CP and 95% after (P< 0.01 per group).

It is therefore clear that an immobilizing stimulus, such as CP, is able to dramatically attenuate both drug and sensory-induced activated behaviors which are thought to be the result of increases in mesencephalic DA function.
SELECTIVE ATTENTION DISRUPTED BY APOMORPHINE IN THE MONGOLIAN GERBIL. Mary Lou Cheal. Neuropsychol. Lab., McLean Hospital, Department of Psychiatry, Harvard Medical School, Belmont MA 02178.

In previous research, selective attention was intact in gerbils following injections of amphetamine (Phys. Behav., 1978, 29, 299-305). Amphetamine-treated (1.0-3.0 mg/kg) selectively responded to objects that were novel even though the amphetamine-induced competing stereotypes resulted in an attenuation of the rate of active investigation of the object. Additionally, when a large dose (6.0 mg/kg) of d-amphetamine was administered, intense stereotypy occurred and very little active investigation (a passive* attitude) could be inferred from data collected the next day when the acute effects of the drug had passed (Soc. Neurosci. Abst., 1978, 4, 731). Because the gerbil is interested in novel objects up to two weeks following a single 60 sec nonreinforced trial (J. Biol. Psychol., 1978, 20, 26-32), it was possible to test amphetamine-injected gerbils 48 hours after injection on the second day. The responses of these gerbils reflected habituation similar to habituation in controls that had actively investigated the object following injection. The stimulus-elicited investigation paradigm allows the separation of active investigation or alertness from selective attention.

Several lines of research implicate the action of amphetamine in increasing dopamine (DA) activity as the mechanism whereby amphetamine affects exploratory behaviors and motor activity. However, in the stimulus-elicited investigation paradigm, the DA receptor stimulant, apomorphine, produced results that differed from those of amphetamine. Selective attention was disrupted following doses as low as 1.0 mg/kg apomorphine. At this dose the gerbils moved to the object as frequently as controls on the first trial, but subsequent trials did not reflect rapid habituation or selection. Although "passive" attention was found following 0.3 mg/kg apomorphine, at higher doses (1.0, 3.0 or 10.0 mg/kg) the gerbils showed no evidence of earlier exposure to the object when tested the second day. It was also demonstrated that failure to habituate following apomorphine injections was not due to amnesia or retrieval problems. Additional gerbils were tested for habituation to the object prior to injections. Following subsequent apomorphine treatment, habituation levels of responding occurred indicating that once the memory was stored, it was not disrupted by apomorphine. The results indicate that the gerbil can disrupt selective attention at doses where active investigation, or alertness, can be alerted. (Supported by the Scottish Rite Shriners' Research Program, N. M. J., U. S. A. and by the Biomedical Research Support Program, D. R. N., N. I. H.)

COCAIN INDUCED STEREOTYPED BEHAVIOR: ONGOING RESPONSES DETERMINE DRUG EFFECTS. Jeremiah P. Collins and Nancy Lesse. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

Stereotyped behaviors result following the administration of a number of different drugs, primarily the psychomotor stimulants. The stereotypies consist of responses occurring repetitively with little or no variation, and appear independent of reinforcement, and are relatively insensitive to changes in ambient stimuli. Examples of these behaviors are repetitive orienting or arousal reactions such as head bobbing, rearing, sitting, grooming, or in man, complex response chains including speech and cognition. Interactions of ongoing behaviors with the actions of psychomotor stimulants have also been reported. We investigate in the present study whether ongoing operant responses occurring at the time of cocaine administration become stereotyped. Cats were trained in a discrimination task in which each bar-press emitted when a tone (S+) was present resulted in milk reinforcement. When the tone was absent (S-) milk was not delivered. The duration of S+ and S- periods varied randomly from 1 to 5 mins and a 1-min DRL contingency was in effect during S-. Training continued until a high level of discrimination was achieved. Drug testing was then begun with injections of cocaine hydrochloride (1 mg/kg). The stereotypic behavior was manipulated by scheduling either a 5-min S+ or S- period immediately following cocaine administration. When the S-period followed cocaine administration, stereotyped bar-pressing developed, i.e. bar-pressing continued during subsequent S- periods despite the absence of milk reinforcement. When the S- period followed cocaine, marked suppression of bar-pressing resulted. This effect was reversible in the same subjects when stimulus conditions were later reversed. A similar study conducted with rats yielded similar results. Whether the mechanisms responsible for these drug-induced behaviors, it is clear that the stimuli and the behaviors occurring at the time of cocaine administration are powerful determinants of subsequent stereotyped behaviors.

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ESTROGEN MODULATION OF DA DEPENDENT BEHAVIORS. L. A. Chiado, A. R. Caggula and C. P. Caggula. Psychology Program, Dept. of Psychology and Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have previously shown that estrogen potentiates two behavioral consequences of drug-induced changes in DA activity. That is, injections of 6-hydroxydopamine (6-OHDA) with estrogen, but not progesterone, increased the number of Dal-pressed (ED; 100 mg/kg, s.c.) 48 h before testing showed significantly longer duration of spiperone-induced catalepsy (0.5 mg/kg) and apomorphine-induced stereotypy (3 and 6 mg/kg) than OVX/controls (Chiado and Caggula, 1978). The present study demonstrates that OVX female rats receiving the same hormone treatment and the same drugs show no significant differences compared to OVX/controls. In all cases stereotypy was rated on a 7 point scale.

We also examined the effects of estrogen on the uptake of radioactively labelled spiperone, amphetamine and apomorphine in order to determine whether estrogen may have increased the behavioral effects of these drugs by altering their peripheral metabolism or uptake into the brain. We now report that an EB treatment which increased spiperone-induced catalepsy (0.5 mg/kg) also elevated levels of 3H-spiperone in both blood and whole brain. EX1 increased whole brain striatum levels, relative to oil controls, at both three (129%) and six hours (129%). Tryptophan levels in blood were also elevated at these same times (11% and 146%). Similar results were obtained after chromatography of brain and blood, indicating that most of the radioactivity was accounted for by 3H-spiperine. These findings suggest that at least some of the increased catalepsy may have resulted from estrogen's ability to increase the levels of spiperone reaching the brain.

In the same effects of EB on the uptake into the brain of tritiated amphetamine (3 mg/kg) and apomorphine (3 mg/kg) administered intraperitoneally. EB did not influence whole brain levels of amphetamine, apomorphine or its active metabolite, p-hydroxydromeridine. Similar results were obtained with chromatography. In fact, it was a non-significant trend for OVX/EB animals to show lower tritium levels than OVX/oil controls. Furthermore, the behavioral effects of amphetamine was not accounted for EB's effects on amphetamine or apomorphine stereotypy.

In summary, both central and peripheral effects of estrogen must be considered when assessing its role in the behavioral and neurochemical consequences of drug-induced alterations in DA function.
was different. There was an initial "psychotomimetic" phase of effects in man. (Supported by Grant DA00376 from NIDA.)

The behaviors typically studied with regard to sensitization in rats; increasing 5-HT by reuptake inhibition (fluoxetine) and administration of precursor (L-tryptophan) blocks muricide in established spontaneous killers. It has been reported that in rats with amygdala lesions induces killing in non-killer rats; increasing 5-HT by reuptake inhibition (fluoxetine) and administration of precursor (L-tryptophan, 5-HTP) and 5-HT agonists (quipazine) blocks muricide in established spontaneous killers. These drugs also increase 5-HT, norepinephrine (NE) and dopamine (DA) by inhibiting MAO, which blocks muricide in established spontaneous killers. The shift was done to test the hypothesis that imipramine and other antidepressants block muricide behavior. These drugs also increase 5-HT, norepinephrine (NE) and dopamine (DA) by inhibiting MAO, which blocks muricide in established spontaneous killers. The shift was done to test the hypothesis that imipramine and other antidepressants block muricide behavior.

PSYCHOPHARMACOLOGY

EFFECTS OF PHENOBARBITAL ON THE ACTIVITY OF HYPERACTIVE AND NON-HYPERACTIVE ANIMALS. J. Diaz, K. Watanabe*, and J. Zagun*. Dept. of Psychology, Univ. of Washington, Seattle, WA 98115, Dept. of Psychiatry, MPR/NI, Los Angeles, CA 90004.

Studies show that stimulants may not only improve the attention span of children with hyperkinetic disorder, but may also reduce their activity. In addition, most central nervous system depressants will not sedate but rather agitate hyperactive children. The purpose of this study was to determine the activity of a CNS depressant - phenobarbital - on the activity of a normally very active rodent - the Mongolian gerbil (Meriones ungulatus) - and a considerably less active rodent - the rat.

Thirty-day old gerbils were assigned to either a phenobarbital group (n=20) or to a vehicle group (n=19) and were injected daily with phenobarbital (60 mg/kg, i.p.) or the vehicle for 4 months. After approximately 14 weeks of drug administration, the activity of the animals in a 5 minute open field test was recorded once a week at varying times after injection (1, 2, 4, 6, 8, 16, 24 hours). During this testing period blood was drawn from the drug animals to determine phenobarbital levels at the time of testing. Vehicle treated animals were subjected to the same procedure to control for the stress of the bleeding. Thirty-day old male Wistar rats were also assigned to either a phenobarbital group (n=20) or to a vehicle group (n=19) and were given drugs and tested in the same way as the gerbils (after 1, 2, 4, 6, 8, 24 hours).

The results indicate that the control gerbils and rats show a stable level of locomotor activity for all time intervals after injections. Moreover, the activity scores for the control gerbils were more than double the activity scores for the control rats. Even though the phenobarbital group showed hyperkinetic activity of rats, it did dramatically alter the activity of gerbils. Animals tested within a few hours after injections were sedated and showed a decrease in activity. The activity level increased for at least 8 hours after injection and the drug group returned to activity levels which were similar to control gerbils. The phenobarbital plasma levels of the drug animals increased even though gerbils clear phenobarbital at a slightly faster rate, both rats and gerbils peak plasma levels at one 24 hours after injections.

The striking similarities between the effects of CNS depressants on the behavior of hyperactive children and the behavior of gerbils. The data from this preliminary study suggest that the gerbil may be a useful model for hyperactivity.


It is believed that the progressive enhancement (sensitization) of behaviors following repeated administration of stimulants such as amphetamine (AM) may mimic the development of AM psychosis in humans, a condition markedly similar to paranoid schizophrenia. Animals have been locomotion and various motor stereotypes. We have shown that the progressive enhancing (sensitization) effect of AM may be seen following only 2 injections of AM spaced at a 24-hour interval.

Rats were injected daily i.p. with saline, 2, 4, or 8 mg/kg of sodium sulfate. The rats were then tested on the 5 minute open field test for 3 days immediately following drug administration on different days into the chronic regimen. No significant drinking occurred acutely. The drinking response is unlikely to be due to conditioning or an accumulation of AM, since the polydipsia could also be seen following only 2 injections of AM spaced at a 20-day interval.

We have yet to address the mechanisms underlying AM-induced drinking, we have observed a significant drinking response which sensitizes during chronic electrical stimulation of the medial forebrain bundle terminal area of the mesocortical dopamine system. Since electrical stimulation of this region also produces a sensitization to AM stereotypy, it is possible that the mesocortical dopaminergic sensitization phenomena in general, as well as in the progressive polydipsia reported here, may also be seen following only 2 injections of AM spaced at a 20-day interval.

We suggest that induced drinking may serve as a suitable measure for studying AM sensitization. The simplicity of the procedure coupled with its objectivity offer distinct advantages over previously employed sensitization paradigms. Finally, it should be noted that the induction of drinking following repeated AM may be clinically relevant, since Bell (1973) has reported increased thirst in individuals manifesting AM psychosis.

PSYCHOPHARMACOLOGY
2196 FOLLOWING SEVERAL DAYS OF CONTINUOUS ADMINISTRATION D-AMPHETAMINE ACQUIRES HALLUCINOGEN-LIKE PROPERTIES. Gaylord Ellison, Erik B. Nielsen, and Arlene Stark. Dept. Psychology, UCLA, Los Angeles, CA 90024

Rats injected with LSD or mescaline show the behavioral syndrome which has been previously reported to be hallucinogen-specific in higher mammals: limb flicks and whole body shakes. Although these behaviors are decreased in a dose-dependent manner by acute injections of d-amphetamine, they are present 4-5 days after rats are implanted with slow-release silicone pellets producing continuous d-amphetamine administration. These behaviors can be elicited at a high frequency in rats which have been pretreated for 4 1/2 days with an amphetamine pellet, given a 12 hr. rest period, and then injected with a low dose of d-amphetamine. Such pellet-pretreated animals also groom their body surface excessively. We propose that this novel behavioral syndrome which follows continuous amphetamine administration can serve as a new animal model of amphetamine psychosis, since it is based on the same drug regimen which most reliably produces a model psychosis in humans. This behavioral syndrome is altered by concurrent injections of dopamine or serotonin antagonists.

2197 EFFECTS OF d-AMPHETAMINE AND NALOXONE ON BRAIN-STIMULATION REWARD. Ralph D. Esposito, William Perry, and Conen Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA. 02118

Rats were stereotaxically implanted with bipolar electrodes aimed at either the medial forebrain bundle or the ventral tegmental area. Self-stimulation thresholds were determined by means of a modification of the psychophysical method of limits (Esposito, R., & Kornetsky, c., Science, 195:189, 1977). Reinforcement values were determined after the administration of d-amphetamine alone, naloxone alone and naloxone administered 2 minutes prior to the injection of d-amphetamine. d-Amphetamine yielded dose related decreases in the threshold (0.25 - 1.00 mg/kg, i.p.), while naloxone alone (2.0 - 16 mg/kg, i.p.) caused no consistent changes. For each animal, the dose of d-amphetamine that yielded the maximum threshold lowering effect was then selected to be administered with varying doses of naloxone. The results indicate that naloxone blocks the threshold lowering effect of d-amphetamine at doses as low as 2.0 or 4.0 mg/kg of naloxone. This finding suggests the possible involvement of an opiate receptor in the mediation of the reward enhancing action of d-amphetamine on brain-stimulation reward.

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2198 STABILIZATION OF BLOOD ETHANOL LEVELS BY SUBCUTANEOUS RELEASE OF ETHANOL IN RATS DRINKING PREFERRED LIQUID DIETS. Carlton K. Erickson, Kathe I. Koch, and Joseph E. Kappeler. Dept. Pharmacol. Coll. Pharmacy, Univ. Texas, Austin, TX 78712

Liquid diets containing ethanol are a popular method of producing long-term physical dependence in rats and mice. The diets consist of formulations developed a) for weight reduction or nutritional supplementation in humans, or b) expressly for rats. We have tested four such formulations: (Lieber-DeCarli, BioServ; Shorey AIN, custom diets) during 5-day preference tests in female Sprague Dawley rats. Ethanol or (in control) dextrin supplied 37% of calories in each diet. Animals were housed individually and liquid diets in sterile bottles equipped with Richter drinking tubes were the sole source of food and water. Fresh diet was provided daily. Rats in general preferred chocolate-flavored diets over vanilla-flavored diets, and dextrin-containing diets over ethanol-containing diets. The Lieber-DeCarli diet with dextrin was preferred over the Shorey AIN diet with dextrin, but when ethanol isocalorically replaced dextrin the preference was reversed. Severe withdrawal symptoms could not be evoked after the 5-day exposure. Blood ethanol levels (BEL) cycling during the diet-drinking periods, as determined by gas chromatographic analysis of arteriovenous (tail) blood samples.

Stabilized BEL (less cycling compared to diet-induced BEL in the above study) were obtained by using diet in combination with subcutaneous silastic tubes (Sustained Ethanol Release Tubes, SERT; Pharmacologist 20: 159, 1978) which slowly released 95% v/v ethanol. These were refilled once a day. An initial intra-gastric dose of ethanol in liquid diet was used to "set" the desired BEL. The SERT sustained the BEL, while an available diet added supplemental ethanol and calories for maintenance of stable BEL and body weight. After 4 days of high BEL (above 200 mg/dl), severe withdrawal symptoms were seen upon removal of ethanol, indicating the development of physical dependence. This combination of two models is therefore useful for studying physical dependence in rats or for studying the effects of stable (or cycling) BEL in rats over short or long-term exposure to ethanol.

(Supported by funds from the Salk Institute-Texas Research Foundation and The University of Texas.)

Numerous studies have indicated that chronic activation or blockade of neurotransmitter receptors may lead to alterations in receptor sensitivity. In a previous study we have reported that the chronic administration of inhibitors of 2-oxoglutarate aminotransferase (GABA-T), the enzyme responsible for the metabolism of GABA in brain, causes an increase in \(^{3} \text{H}\)-spiroperidol binding, a decrease in \(^{3} \text{H}\)-muscimol binding but no change in cholinergic muscarinic receptor binding in homogenates of rat corpus striatum (Proc. Fed. Proc. 38:767, 1979). Kinetic analysis of ligand binding indicated that these changes were due to alterations in receptor number but not receptor affinity. The increase in dopamine receptors correlated with an increase in stereotypic behavior following an apomorphine challenge in mice treated chronically with a GABA-T inhibitor. In the present investigation, studies were undertaken to further study neurotransmitter receptor interactions in this brain region following chronic administration of drugs. Rats were administered either aminooxyacetic acid (AAOA) (10 mg/kg, i.p.), atropine (At)(1 mg/kg, i.p.) or AOA (10 mg/kg i.p.) and At (1 mg/kg, i.p.) for 15 days, and 24 hr following the last injection the brains were removed, dissected and the corpus striatum was homogenized. In agreement with previous results, AOA caused an increase in \(^{3} \text{H}\)-spiroperidol binding in the corpus striatum of treated animals. However, in the At and At and AOA treated animals, \(^{3} \text{H}\)-spiroperidol binding was significantly decreased (38%) with respect to control animals. These findings suggest a cholinergic influence on dopamineergic activity in this brain area. In addition studies, animals were treated chronically with ethanol alone (30%) (500 mg/kg i.p.), a peripheral inhibitor of GABA-T, muscimol (0.6 mg/kg i.p.) or a direct GABA receptor agonist, or ESO (500 mg/kg, i.p.) plus Mus (0.6 mg/kg, i.p.). At the doses studied, neither GABA nor dopamine receptor binding was significantly altered in the corpus striatum of any of these treatments. This finding suggests that, at this dose, systemically administered muscimol may not act as a GABA receptor agonist in this region of the corpus striatum. The changes in dopamine receptor binding studies may be useful for investigating neurotransmitter interactions in brain and may provide information about receptor sensitivity. (Supported in part by USPHS grants NS-13803, HE-07688 and an RCDA NS-00335 (S.J.E.).)

ALTERATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF ETHANOL DURING CHRONIC ETHANOL TREATMENT. S. Glisky and H.L. Altshuler. TRIMS and Baylor College of Medicine, Texas Medical Center, Houston, TX 77030

This study evaluated the effects of chronic ethanol (ETOH) administration on the discriminative stimulus properties of ETOH in rats trained to perform a double lever operant drug discrimination task. Twenty-five male Sprague-Dawley rats ranging in weight from 240 to 260 gm were used in these studies. These were reduced to 80 per cent of free feeding weight and gradually shaped to perform operant drug discriminations. After the rats were shown to perform at least 70 per cent correct responding at their respective ETOH dose levels, a variety of schedules culminating in the performance of the double lever choice paradigm during a DRL-10 schedule of reinforcement. The animals were then shaped to preferentially respond on the lever when they had previously been paired with ETOH (0.100 mg/kg, IP, 15 min. prior to testing). Criteria for correct responding in this portion of the shaping schedule was that the group mean ETOH correct lever responses was at least 80% of total responses. Following acquisition of this behavior at the ETOH training dose was gradually reduced from 1.0 mg/kg, IP to 0.75 mg/kg, IP during a 30 day period. Following acquisition of the 0.75 mg/kg discrimination, a dose response curve was generated to establish the generalization of correct discriminations across a range of ETOH doses from 0.5 mg/kg-1.5 mg/kg. The animals were divided into three groups, Group A received saline chronically. Group B, ETOH, 2.5 mg/kg, three times daily and Group C ETOH 5 mg/kg, three times daily. Drug was administered by intragastric intubation, 7 days per week. The animals were tested every ten days to assess levels of correct responses after saline or ETOH (0.75 mg/kg, IP) pair treatment. Chronic ETOH administration produced significant reductions in the discriminative stimulus properties of ETOH at all points in the dose response curve, producing a highly significant shift to the right of the DRL-10 schedule of reinforcement. The differences in the four-way daily and dose and response, generalization curves were observed after 30 days and 60 days. These experiments demonstrated that chronic ETOH administration produced significant reductions in the discriminative stimulus properties of ETOH at all points in the dose response curve, producing a highly significant shift to the right of the DRL-10 schedule of reinforcement. In general, differences in the four-way daily and dose and response, generalization curves were observed. No significant changes in correct responding were noted in the saline control group. This data demonstrate that the chronic administration of ETOH to rats trained to perform a double lever operant task produced dose related alterations in the discriminative stimulus properties of ethanol, suggesting that the development of tolerance to ethanol is associated with reduction in the animal's perception of the drug's effects.

Male Sprague-Dawley rats were stereotaxically implanted with bipolar electrodes aimed at various self-stimulation sites. After recovery from surgery, all rats were shaped to work for 1.0 mg/kg morphine sulfate in a pair of -conditioning pulses. The first pulse in a pair of conditioning pulses was followed by a second (X-test pulse) which was parametrically varied in time (1.5-5.0 msec). A condition in which the T-pulse was omitted was also randomly presented with these values in a Latin square design.

All subjects were then tested until ICS response rates were stable. These animals were then entered into a drug paradigm of 3 days (1.5 mg/kg saline followed by a fourth injection day of naloxone hydrochloride). The dosage of naloxone to be administered (1, 10, 10, 0, or 40 mg/kg) was determined by a counterbalanced design. This sequence of naloxone and saline was repeated until all animals completed three tests at each dose level. All subjects were then given three more days of each dose and by a fourth injection day of morphine at 1.25 mg/kg. Naloxone was found to differentially affect ICS response rates. The subjects demonstrated response suppression at the higher ICS response rates at the higher concentrations and remained the same under naloxone. Thus far, subjects showing a response suppression have enhanced their morphine response rates, while subjects showing no naloxone induced rate suppressions have evidenced no effect to morphine. The data also suggest a reversible tolerance to naloxone (progressive sensitivity) from some sites.


We have previously used a sensitive behavioral procedure to define the antipunishment (anticontlict) effects of benzodiazepine anxiolytic agents (JPET 204: 88, 1976) and of barbiturates (Fed. Proc. 37: 617, 1978). In the present experiments, the benzodiazepine compound, flunitrazepam (FLNZT), the antidepressant agents, amitriptyline (AMIT) and maprotiline (MAPR), and the antipsychotic agent, clozapine (CLOZ), were studied. In addition, the a-agonistic antagonist propranolol (PROP), which has been claimed to have some anxiolytic activity clinically, was studied alone and in combination with clozapine (DIAZ) or chloridiazepoxide (CDAP). Compounds were administered by intragastric tube (three to eight monkeys per dose level) 30 min. before a 90 min. test session. Twenty-one squirrel monkeys working on a food-maintained two-lever concurrent VI 1.5' VI 6' schedule. Responses in the VI 1.5' component was also punished intermittently (VR 24) with footshock.

Like other benzodiazepines, FLNZT increased responding in the punished component (Min. Effect. Dose = 0.04 mg/kg), but was 8 and 16 times more potent than DIAZ and CDAP, respectively; however, FLNZT was effective over a narrower range of doses. In agreement with its clinical efficacy as an hypnotic agent, FLNZT at 0.62 mg/kg appeared to induce sleep rapidly in 5 out of 6 monkeys. When tested 24 hrs. later, these animals had recovered to baseline levels of performance. CLOZ exhibited significant anticonflict activity of a low magnitude within a limited dose range (1.25 to 2.5 mg/kg). At 5 and 10 mg/kg, dose-related depression of both punished and unpunished responding was seen; in some monkeys these effects persisted for at least 24 hours. Neither AMIT (0.62 to 10 mg/kg) nor MAPR (2.5 to 20 mg/kg) showed any anticonflict activity. At 10 mg/kg AMIT decreased both punished and unpunished responding although no overt symptoms of sedation were seen. At 20 mg/kg, MAPR decreased responding in some monkeys and produced copious salivation. Thus, neither antidepressant agent exhibited anticonflict-like activity in this test up to doses that produced side-effects.

PROP (1.25 to 40 mg/kg) showed no anticonflict activity; depressed responding and salivation was observed at 40 mg/kg. Nor did PROP (10 mg/kg) show any additive or potentiative effects when it was given in combination with either 2.5 mg/kg of DIAZ or CDAP. These results are consistent with previous animal experiments suggesting that the anxiolytic effects of propranolol, if real, are of a different kind than those of benzodiazepines.
Nutritionally matched pregnant female rats received ethanol or sucrose in liquid diets from the third or 15th day of gestation. Another group received ad lib lab chow and water. Litters were reduced to eight and cross-fostered on the third day after birth. Thus, there were groups whose mothers had ethanol diets during gestation and lactation, or during gestation only, or during lactation only. Animals born to mothers receiving the ethanol diet or the sucrose diet were smaller and lighter than the offspring of the ad lib lab chow mothers. Developmental patterns of activity indicated all groups except the ad lib lab chow group showed abnormal development, and no increased activity in the experimental offspring was found. The weight and activity effects are explainable as malnutrition effects or as being due to the confounding of ethanol exposure and malnutrition. Offspring of animals receiving the ethanol diet showed an increased ethanol preference at 20 days of age, an effect not explainable by malnutrition effects. At 70 days of age all groups showed reduced ethanol preference, especially those whose mothers received ethanol diets. Additionally, males showed a greater reduction in ethanol preference than did females.

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MOTOR RESPONSE TO AMPHETAMINE (Ervin et al., Brain Res. 132:507, 1977) and with decreased activity in the open field (Fink et al., Neuroscience Abstract 660, 1976). Mice injected with morphine sulphate (25- or 50-mg/kg) or etorphine hydrochloride (0.5-mg/kg) showed a typical opiate syndrome characterized by Straub tail and compulsive, robot-like ambulation around the perimeter of the glass jar. These opiate-induced mice were completely unresponsive to morphine and etorphine. The opiate behavioral syndrome was fully reversed by naloxone at 1-mg/kg. These results emphasize the strikingly different responses to opiate by different species (as noted previously by other workers).

The present results suggest that naloxone in vivo in mice has two distinct effects: (1) It antagonized the behavioral effects of opiates at low doses; (2) It inhibited behavior arising from the "emotional" effects of being placed in an unfamiliar environment. This latter higher dose of naloxone, being only partially effective at the low dose. At the highest dose (100-mg/kg), this "sedative" action of naloxone resulted in an effect similar to previously reported for naloxone. Both actions of naloxone were stereospecific, occurring only with the (+-)-enantiomer, and not with the (-)-enantiomer.


Severe bilateral denervation occurs in all dopaminergic DA mesolimbicocortical terminal fields. Bilateral injection of 6-hydroxypamine (6-OHDA) into the anterolateral hypothalamus (Pint, Greenfield, and Smith, Neuroscience Abstract 660, 1976). Bilateral denervation was also correlated with a decrease in locomotor response to amphetamine (Ervin et al., Brain Res. 132:507, 1977) and with decreased activity in the open field (Fink et al., 1976). To determine if bilateral denervation is necessary to produce these behavioral deficits, we injected 6-OHDA into right (n=4) or left (n=4) anterolateral hypothalamus (K7.0, L2.0, 8.0 mm down from the groove). Vehicle-injected animals were made to the right (n=4) or left (n=3) anterolateral hypothalamic site. 6-OHDA damage of ascending NE fibers was prevented by pretreatment with DE. Behavioral tests began 15 days after surgery. Rats were tested in the open field (OF) for 5 min on 3 consecutive days. 6-OHDA rats traversed less than half the number of squares vehicle rats traversed on all 3 days (p<.05). Right and left 6-OHDA injections produced the same results. After OF testing was completed, 6-OHDA and vehicle rats were injected with d-amphetamine (1.5 mg/kg) and the locomotor response to amphetamine was recorded by interruptions of photocell beams for 2 hours. The locomotor response of 6-OHDA rats was about 50% of the locomotor response of vehicle rats (p<.05). No circuling was observed and right and left 6-OHDA injections produced the same results.

The significant decrease in activity in the OF and in response to amphetamine observed in unilateral 6-OHDA rats demonstrates that bilateral denervation of any one mesolimbicocortical DA terminal field is not required for significant decreases in activity to novel stimuli of an OF and to a low dose of amphetamine. The fact that unilateral 6-OHDA rats responded significantly more to amphetamine than bilateral 6-OHDA rats supports the hypothesis that mesolimbicocortical DA denervation correlates with the magnitude of the decrease of the dopamine response. All the results are consistent with an unusual function of the mesolimbicocortical DA system for locomotor responses to a novel OF and to a low dose of amphetamine.

REDUCED PHYSIOLOGICAL AND SUBJECTIVE EFFECTS AFTER REPEATED ADMINISTRATION OF COCAINE IN HUMANS. J. J. Javaheri. M. H. Fischer, R. P. Davis and C. F. Schugardt. Illinois State Psychiatric Institute, IL 60612 and Department of Psychiatry, University of Chicago, IL 60637

Cocaine is a potent central nervous system stimulant and local anesthetic. Although, it is believed that cocaine use produces psychological dependence, most studies suggest that there is no tolerance developed to its psychoactive effects. In fact, it has been suggested that repeated use of cocaine devlops supersensitivity to its behavioral and physiological effects. In this study, cocaine HCl was administered intranasally followed by intravenous injection. Eight volunteers, who had given their informed consent, were included in the study. Each received, either 4 mg or 36 mg of cocaine HCl, intranasally. Sixty minutes later, either 16 mg, 32 mg or 48 mg of cocaine were given intravenously. Blood was withdrawn for cocaine determinations prior to the drug administration and at different time after drug administration. Physiological measures were monitored continuously and subjective effects of the drug were measured by the stimulant sections of the Addictive Research Center Inventory (ARC) questionnaire and the Profile of Mood Scales (POMS).

The plasma concentrations of cocaine were always related to dose administered. This was not true for changes in heart rate and subjective effects. When intravenous cocaine was given after 96 mg intranasal administration the increase in heart rate was not as great as when an intravenous injection followed 4 mg inhalation. Comparable changes were observed in subjective effects. The heart rate changes were plotted against plasma concentrations of cocaine. Unlike an intravenous injection, an intranasal injection showed low dose inhalation. These results suggest that there is a decrease in physiological and subjective effects of cocaine when administered repeatedly in humans. (Supported in part by PHS grant DA01491)

EFFECTS OF NEONATAL LEAD EXPOSURE ON SPONTANEOUS ALTERTATION PERFORMANCE IN RATS. Daniel L. Jones. Dept. of Microscopic Anatomy Baylor College of Dentistry, Dallas, TX 75246

One frequent consequence of subacute lead toxicity is hyperactivity, usually viewed as a result of altered calcium metabolism and/or lack of habituation to environmental stimuli. Spontaneous alternation performance is a behavioral measure which can be used to ascertain the status of this alternation performance is thought to be a result of habituation to the cues of the maze arm most recently visited, leading to exploration of the cues of the arm least recently visited. The effect of neonatal lead exposure on alternation performance, is to decrease the number of alternation of the maze arm least recently visited. The apparatus used for these measurements is a symmetrical T maze. The sequence of arm entries as well as the total number of entries is noted, and a percentage alternation score is calculated. An alternation score is defined as the animal's leaving one arm of the maze and entering the least recently visited of the other two.

Accordingly, the effect of neonatal lead exposure on spontaneous alternation performance in nature albino rats (N=16) was studied. The lead was delivered orally in a concentration of 225 mg/kg body wt, from day 1 following birth through day 20. The lead solutions were made up each day so that the appropriate dosage of lead was delivered in a 0.0025 ml/kg volume of distilled water. Beginning on day 70, the animals were tested daily in 15 minute sessions. After completion of the testing program, the animals were killed by CO2 asphyxiation. Physiological determinations prior to the drug administration and at different time after drug administration. Physiological measures were monitored continuously and subjective effects of the drug were measured by the stimulant sections of the Addictive Research Center Inventory (ARC) questionnaire and the Profile of Mood Scales (POMS).

The plasma concentrations of cocaine were always related to dose administered. This was not true for changes in heart rate and subjective effects. When intravenous cocaine was given after 96 mg intranasal administration the increase in heart rate was not as great as when an intravenous injection followed 4 mg inhalation. Comparable changes were observed in subjective effects. The heart rate changes were plotted against plasma concentrations of cocaine. Unlike an intravenous injection, an intranasal injection showed low dose inhalation. These results suggest that there is a decrease in physiological and subjective effects of cocaine when administered repeatedly in humans. (Supported in part by PHS grant DA01491)

Supported by NIMH Grants MH08402 and MH00149.
ENHANCED ACUTE TOLERANCE IN RATS TREATED CHRONICALLY WITH OPIATES.


Tolerance occurs in naive subjects during the action of a single opiate injection. The present study suggests that such acute tolerance is important for the understanding of chronic tolerance.

In Expt. 1, male Wistar rats treated with morphine-S04 (MS) for 49 days (0, 20 or 200 mg/kg maintenance doses) were tested for analgesia (tail immersion test; 48°C water) after i.p. etorphine-HCl (2.4-6.6 μg/kg for 20 and 200 mg/kg groups). Tests at 0.5 hr intervals showed faster within-session loss of analgesia in chronically tolerant than non-tolerant rats. In Expt. 2 these effects were replicated in similarly treated rats after injection of etorphine into the lateral ventricle (0.1-5 μg, 0.24-4 μg, and 0.6-10 μg/rat for 0, 20 and 200 mg/kg MS groups respectively).

The rapid acute tolerance was not due to repeated testing. Rats treated chronically with MS, 20 and 200 mg/kg, received either saline or MS (10 mg/kg i.v.) pretreatment, followed 1 hr later by i.v. injection of 25 or 50 mg/kg MS and a single tail-flick test 2 min later. Latencies were 50% lower in the MS pretreated than in saline pre-treated rats. In contrast, MS pretreatment in chronic saline controls increased the latencies slightly. Similarly, pretreatment with MS (30 μg into the III ventricle), in rats treated chronically with etorphine (200 μg/kg twice daily), reduced the analgesia at 30 min after a test dose of etorphine (200 μg/kg i.p.).

Thus, a very rapid acute tolerance was found in chronically opiate-treated rats. Earlier, we reported (Psychopharmacology, 1978, 60, 59-65) similar findings in ethanol experiments. We suggest that chronic tolerance is an enhancement of acute tolerance. This effect may be critical for estimating treatment-produced changes in opiate sensitivity; log dose/response (LDR) curves for data from Expt. 1 and 2 indicated a tolerance-related shift to the right at short test intervals and shift plus flattening at intermediate intervals. In addition, the nature of the acute tolerance suggested that it could be used to localize sites in the brain responsible for high levels of tolerance to systemically administered opiates, and for the development of receptor models.

DIETARY TRYPTOPHAN MODULATION AND AGGRESSIVE BEHAVIOR IN MICE.

Kathleen M. Kantak, Linda R. Hegstrand and Burr Eichelman. Univ. of Wisconsin and William S. Middleton Veterans Medical Center, Madison, WI 53706.

The effects of a tryptophan-free diet on isolation-induced fighting and predatory cricket killing in mice were examined. The results demonstrated that consumption of a tryptophan-free diet for 18 days 1) decreased both the number of fighters and duration of isolation-induced fighting; 2) increased the number of cricket-killing mice and decreased the latencies to attack and to kill crickets; 3) reduced brain serotonin 27%; b) increased water intake 38%; and c) decreased body weight 27% without affecting food intake. To determine if these effects were due specifically to the lack of dietary tryptophan, other groups of mice were fed a 5% tryptophan load in the standard chow; a 0.15% tryptophan supplement in the tryptophan-free diet; or a 3 grams/day calorically-restricted chow diet. The lack of tryptophan in the diet specifically produced the almost total inhibition in isolation-induced fighting, the reduction in brain serotonin, and the large decrease in body weight. The other non-specific effects appeared to be related to general factors such as dietary need for the cricket killing diet or diet composition for the water intake.

ROLE OF THE PERIAQUADDUCTAL GRAY IN THE DISCRIMINATIVE STIMULUS PROPERTIES OF METHODENE.


Rats, trained to discriminate methedone injections (1.5 mg/kg, s.c.) from saline injections on a standard two-bar operant task, were assessed for generalization to central administration of methedone and morphine at the site of the periaqueductal gray (PAG). Initially, animals were trained to discriminate following methedone injections (1.5 mg/kg) and on the opposite lever following saline (1 ml/kg) injections during the 15 min daily 7 sessions. Reinforcement was delivered on a variable interval (VI)-15 sec. schedule for correct-lever responding (0.1 ml sweetened milk). Training continued until discrimination of each drug state was reliable when assessed during 2 min extinction sessions. Extinction sessions for generalization testing occurred once a week with continued training under both drug states between generalization tests. Subsequent to discrimination training, animals were anesthetized (40 mg/kg Nembutal) and implanted with stainless-steel (24 ga.) cannulae in the PAG for direct drug injection. Surgical recovery and relocalization on the VI-15 sec. schedule were followed by testing for generalization to the methadone cue. PAG injections were accomplished by removal of a 31 ga. needle which extended 1 mm beyond the guide cannulae. All drug doses tested were suspended in a total volume of 1 ul saline for injection. Both dose response and time course data were collected following the direct application of morphine and methadone at the site of the PAG.

Peripheral injections of methadone produced 89.1 ± 6.7% drug-lever responding and morphine injections 5.1 ± 7.2% drug-lever responding prior to generalization testing at the PAG site. The peripherally administered methadone training cue could not be mimicked by PAG injections of methadone (0, 10, 20 or 40 μg/rat) at any of the time parameters examined (0, 15 or 30 min postinjection). Although the PAG was insensitive to methadone, direct morphine application at the site of the PAG did generalize the methadone training cue (1.5 mg/kg) at a mean dose of 3.8 ± 1.9 μg. These findings suggest that morphine and methadone produce similar discriminable cues but the role of the PAG in the methadone cue is not clear. The PAG has been implicated in the action of morphine and in the discriminable cue of morphine but the discriminable cue of methadone may be dependent on other central sites. (Supported by U.S.P.H.S. grant DA-00296-05).

LITHIUM DECREASES SELF-STIMULATION AND ESCAPE IN RATS INDEPENDENT OF LOCOMOTOR ACTIVITY.


The effects of lithium chloride (2 mg/kg) on self-stimulation and escape behavior elicited by intracranial electrical stimulation of the medial forebrain bundle and the midbrain reticular formation respectively, were studied and tested in chronically implanted rats. In the same test periods, locomotor activity was also measured. A "hit-box" method allowed the animals to control the current of brain stimulation (0, 20 or 40 μA). Post-drug performance was followed over up to four hours. Throughout, data was tabulated at 8 min. intervals. In 7 experiments (4 rats), pre-drug self-stimulation increased activity compared to periods without brain stimulation; and lithium caused both self-stimulation and activity to decrease. In 7 escape experiments (4 rats), lithium decreased escape although locomotor activity was not changed. At higher stimulus currents eliciting better performance, both self-stimulation and escape were less affected by this dose of lithium. Thus, lithium may be acting to a significant extent directly on affective mechanism of the brain, and may not be merely sedative in its action, since escape decreased while locomotor activity did not. (Supported by NIH Grant MH-08102.)

Previous work has established that the administration of tyrosine (6-OHDA) lesions to the nucleus accumbens septi (NAS) produce transient hypoaactivity and block the increase in locomotor activity induced by L-5-hydroxytryptophan (L-5-HTP) in rats. L-5-HTP is known to produce a selective decrease in tyrosine hydroxylase (TH) activity in the ventral tegmental area (VTA) to induce a significant hypertonia and fail to block AMPH induced locomotor activity. At the AMPH dose was used to determine the specificity of this effect. Rats pretreated with pargyline (50 mg/kg) were injected intracerebrally with 6-OHDA and the locomotor response was measured by a significant increase in locomotor activity. The rats showed a block of AMPH activity identical to that observed with the NAS lesion alone. All lesion groups that showed a block of AMPH locomotor activity exhibited supersensitivity to AMPH as measured by a significant increase in locomotor activity. The rats treated with AMPH showed similar results.

The inhibition of the mesolimbic dopamine system can produce hypertonia but that more extensive destruction of this system produces no hyperactivity and an attenuation of the locomotor response to AMPH. These results suggest that limited destruction of the mesolimbic dopamine system can produce hypertonia but that more extensive destruction of this system produces no hyperactivity and an attenuation of the locomotor response to AMPH.


Previous studies from our laboratory have shown that drug treatments which interfere with catecholamine metabolism can significantly alter the response of group assembled monkeys to separation from their peer group, in the absence of demonstrable drug effects on group social behavior (F. G. Koob, L. Stinus, M . Le Moal, D.C.S. Roberts, and F.E. Bloom, A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037 and Laboratoire de Neurobiologie des Comportements, Universite de Bordeaux, Talence, France).

The administration of tyrosine via the diet has been reported to decrease spontaneous locomotion and brain dopamine concentrations in mice (Lasley et al. Neurosci. Abstr., 1970). Employing a resident-intruder aggression test, we examined the effects of dietary tyrosine (10% of the diet) on tyrosine concentrations in the neostriatum and brain dopamine concentrations in mice. Treatment with 6-OHDA lesions alone failed to significantly alter activity. 6-OHDA lesions to the NAS produced a block of AMPH activity that was directly related to the dose of 6-OHDA used with the 4ug/1ul dose producing a 90% decrease. The posterior and medial NAS lesions produced an identical block of AMPH activity. The rats with the RF lesions to the VTA were spontaneously hyperactive and remained hypertonic after injection of AMPH. In contrast, the rats with the combined NAS (RF) or 6-OHDA lesions showed a block of AMPH activity identical to that observed with the RF lesion alone. All lesion groups that showed a block of AMPH locomotor activity exhibited supersensitivity to APO as measured by a significant increase in locomotor activity. The rats treated with AMPH showed similar results.

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In order to investigate whether anti-psychotic drugs inhibit the binding of [3H]-spiperone to neuroleptic/dopamine receptors in human brain in accordance with their clinical potencies, we determined the effect of 22 different neuroleptics on the specific binding of [3H]-spiperone to caudate and putamen of post-mortem human brain.

All clinically active neuroleptics effectively inhibited the binding of [3H]-spiperone. β-flupenthixol, trans-thiothixene and (-)-butaclamol were ineffective even at micromolar concentrations. The potencies of these drugs for inhibiting [3H]-spiperone binding varied well with their clinical potencies for treating schizophrenia. The 50% inhibitory concentrations (IC50) of these drugs were as follows:

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>IC50 nM</th>
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<tbody>
<tr>
<td>Spiperone</td>
<td>0.3</td>
</tr>
<tr>
<td>Benperidol</td>
<td>0.3</td>
</tr>
<tr>
<td>Trifluperid</td>
<td>0.3</td>
</tr>
<tr>
<td>Pimozide</td>
<td>3</td>
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<tr>
<td>Haloperidol</td>
<td>3</td>
</tr>
<tr>
<td>Mesulprimine</td>
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<tr>
<td>Noperone</td>
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<tr>
<td>Leperone</td>
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<tr>
<td>(+)-butaclamol</td>
<td>0.3</td>
</tr>
<tr>
<td>(-)-butaclamol</td>
<td>0.3</td>
</tr>
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<td>Fluphenazine</td>
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</tr>
<tr>
<td>Trifluromazine</td>
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<td>Trifluoperazine</td>
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<tr>
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</tr>
<tr>
<td>Promazine</td>
<td>3-5</td>
</tr>
<tr>
<td>Carbamol</td>
<td>10</td>
</tr>
<tr>
<td>GABA</td>
<td>10</td>
</tr>
</tbody>
</table>

It remains to be determined whether such a profile also holds in schizophrenic brain tissues where higher numbers of neuroleptic receptors have been found (Lees and Seeman, Proc. Soc. Neurosci. 1973, 1978). Scatchard analysis using [3H]-spiperone indicated that there was no change in receptor affinity in schizophrenic tissues compared to those from normal controls. (Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada.)

**PSYCHOPHARMACOLOGY**

**CORRELATION OF ANTI-PsyChotic DRUG POTENCY AND NEUROLEPTIC RECEPTOR INHIBITION IN POST-MORTEM HUMAN BRAINS.**

Tyronne Lee and Philip Seeman. Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

In order to investigate whether anti-psychotic drugs inhibit the binding of [3H]-spiperone to neuroleptic/dopamine receptors in human brain in accordance with their clinical potencies, we determined the effect of 22 different neuroleptics in inhibiting the specific binding of [3H]-spiperone to caudate and putamen of post-mortem human brain.

All clinically active neuroleptics effectively inhibited the binding of [3H]-spiperone. β-flupenthixol, trans-thiothixene and (-)-butaclamol were ineffective even at micromolar concentrations. The potencies of these drugs for inhibiting [3H]-spiperone binding varied well with their clinical potencies for treating schizophrenia. The 50% inhibitory concentrations (IC50) of these drugs were as follows:

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>IC50 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiperone</td>
<td>0.3</td>
</tr>
<tr>
<td>Benperidol</td>
<td>0.3</td>
</tr>
<tr>
<td>Trifluperid</td>
<td>0.3</td>
</tr>
<tr>
<td>Pimozide</td>
<td>3</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>3</td>
</tr>
<tr>
<td>Mesulprimine</td>
<td>3</td>
</tr>
<tr>
<td>Noperone</td>
<td>4</td>
</tr>
<tr>
<td>Leperone</td>
<td>10</td>
</tr>
<tr>
<td>(+)-butaclamol</td>
<td>0.3</td>
</tr>
<tr>
<td>(-)-butaclamol</td>
<td>0.3</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1</td>
</tr>
<tr>
<td>Trifluromazine</td>
<td>1</td>
</tr>
<tr>
<td>Prolchlorperazine</td>
<td>1</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>1</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>1</td>
</tr>
<tr>
<td>Promazine</td>
<td>3-5</td>
</tr>
<tr>
<td>Carbamol</td>
<td>10</td>
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**BEHAVIORAL AND BIOCHEMICAL STUDIES OF AMPHETAMINE TOLERANCE AND REVERSE TOLERANCE.**

Nancy J. Leith & Ronald Kuczenski, Dept. Pharmacol., Vanderbilt School of Medicine, Nashville, TN 37232

Leith & Barrett (Psychopharmac., 48, 1976) have demonstrated that following chronic administration of d-amphetamine, (increasing doses, 3 times daily for 4 days) tolerance is seen to the facilitating effects of the drug on self-stimulation responding. On the other hand, Segal & Mandell (Pharmacol., Biochem. Behav. 2, 1979) showed that repeated administration (constant dose once daily for 7-19 days) of the drug results in an enhanced response producing effects. These data suggest that the drug effects on self-stimulation responding and motor behavior reflect different actions of amphetamine. However, the chronic regimens used in the two studies were quite different and could have attributed to the dissimilar results obtained. The present study compared both drug regimens on both behaviors to determine which interpretation is accurate.

For the motor activity studies, male Sprague-Dawley rats were injected with 0.125, 0.25, 0.5 or 1.0 mg/kg d-amphetamine and activity monitored for 3 hours. Half of the animals in each dosage group then received 3.0 mg/kg daily for 6 days and were retested on day 7. The other half received 3 injections daily for 4 days beginning with 1 mg/kg and increasing by 1 mg/kg at each injection and were retested on day 7. Regardless of the chronic injection regimen, all animals showed an enhanced response to d-amphetamine when retested. Saliva production from all animals were implanted with electrodes in the MFB and after training, were tested with an acute dose of 0.3 mg/kg. The animals were then treated chronically as stated above. Animals receiving 3 mg/kg daily showed no change in the amphetamine response when retested whereas those treated 3 times daily with increasing doses demonstrated tolerance as previously reported. In summary, the two behaviors do in fact reflect different aspects of amphetamine's action.

As a first step in assessing possible biochemical changes that might underlie the behavioral phenomena occurring after chronic administration of amphetamine, we have measured DOPAC and HVA levels in the caudate and mesolimbic area. The chronically treated animals showed a significant decrease compared to controls for both DOPAC and HVA in the caudate but not the mesolimbic region when killed 48 hours after the last drug injection, but the two groups were not significantly different from each other at a challenge dose of 3 mg/kg d-amphetamine. In addition, in an attempt to further characterize the nature of the changes in dopamine function, the responses to Haloperidol and apomorphine were being evaluated in animals treated chronically with amphetamine.

(Supported by NIH Grants MH29217 and MH29106 and MH00091.)
The rate dependency hypothesis states that baseline rates of responding determine the effects of amphetamine on schedule-controlled operant behavior. However, the correlation between response rate and reinforcement rate under most reinforcement schedules confounds attributing the schedule-dependent effects of amphetamine solely to differences in rates of responding. Two comparisons were performed to independently evaluate the roles of response rate and reinforcement rate in the schedule-dependent effects of amphetamine. The comparisons were complementary, in that multiple schedules were arranged where one variable, either response rate or rate of reinforcement, was held constant and the other variable was allowed to vary.

In the first comparison, a procedure yoked reinforcement availability under a variable-interval (VI) schedule to the temporal pattern of reinforcement under a differential reinforcement of low rate (DRL) schedule (mult DRL 7 sec yoked VI). VI response rates were 3.2 times greater than DRL response rates, at rates of reinforcement that were held relatively constant. Amphetamine increased DRL responding at the same doses that decreased VI responding. This agrees with previous demonstrations of rate-dependent amphetamine effects when these schedules were studied individually, but further indicates that different rates of reinforcement between schedules are not necessary to produce schedule-dependent amphetamine effects.

The second comparison examined the hypothesis that different reinforcement rates could produce independent effects on amphetamine action. A multiple random ratio (RR) schedule (mult R820 RR50) held rates of responding constant while rates of reinforcement varied by 2.7-fold. Amphetamine affected responding under both ratio schedules equally, indicating that the different reinforcement rates exerted no effects on responding under amphetamine.

The results of the complemented procedures agreed in assessment of the rate dependency hypothesis. The primary determinant of amphetamine's schedule-dependent behavioral effects is the control rate of responding, rather than the control rate of reinforcement.
The interactions between the "dissociative anesthetics", phencyclidine (PCP), ketamine (K), and the antipsychotic drugs, fluphenazine (P), haloperidol (H), α-flupenthixol (αF) were studied in the cerebellum of urethane-anesthetized rats. The latency for the depressions were 2 to 3 seconds and recovery was seen 2 to 3 seconds following termination of drug ejection. Ketamine was one of the first active compounds as PCP and K on cell discharge whereas αF was ineffective. P, H and chlorpromazine also antagonized the depressant effects of PCP and K on P cell discharge whereas β-flupenthixol was used as a control. Activity was studied in the cerebellum of urethane-anesthetized rats. The norepinephrine (NE) and locus coeruleus stimulation on these neurons was by far the more potent of the two compounds, producing a 71% latency for the depressions was 2 to 3 seconds and recovery more than 3

These results taken together with previous reports that antipsychotic drugs block noradrenergic transmission in the cerebellum and that the noradrenergic neurotransmission in the cerebellum and that the cerebellar noradrenergic pathway is a convenient model system to study the effect of ouabain (10-5M) or unlabeled AMPH isomers inhibited d-AMPH accumulation in the striatal preparation. Thus at 37°, little or no effect of ouabain (10-5M) was observed on d-AMPH. Desipramine (10-10-6M) or unlabeled AMPH isomers inhibited d-AMPH accumulation more than 1-AMPH, but more meaningful was the finding that 3-AMPH accumulation was inhibited equally by unlabeled d- or 1-AMPH (10-6M) as was 3-AMPH accumulation. These preliminary findings are suggestive of the fact, unlike striatal DA neurons, little active accumulation or stereoselectivity of binding of AMPH isomers exists in the case of hypothalamic NE neurons. The findings consistent with other known electrophysiological and biochemical equipotency of AMPH isomers on NE neurons as contrasted with noradrenergic DA neurons where the d-isomer is much more potent than the l-isomer. (Supported by USPHS Grant MH-05831.)

In the present study, similar experiments were performed using a crude synaptosomal preparation of rat hypothalamus, an area rich in NE nerve terminals. Accumulation of 3H-AMPH (10-7) was observed at 37°, but while this was inhibited by cold, the pattern of inhibition by drugs differed markedly from that seen in the striatal preparation. Thus at 37°, little or no effect of ouabain (10-5M) was observed on d-AMPH. Desipramine (10-10-6M) or unlabeled AMPH isomers inhibited d-AMPH accumulation more than 1-AMPH, but more meaningful was the finding that 3-AMPH accumulation was inhibited equally by unlabeled d- or 1-AMPH (10-6M) as was 3-AMPH accumulation. These preliminary findings are suggestive of the fact, unlike striatal DA neurons, little active accumulation or stereoselectivity of binding of AMPH isomers exists in the case of hypothalamic NE neurons. The findings consistent with other known electrophysiological and biochemical equipotency of AMPH isomers on NE neurons as contrasted with noradrenergic DA neurons where the d-isomer is much more potent than the l-isomer. (Supported by USPHS Grant MH-05831.)

Administration of 10.0 mg/kg imipramine (IM, 1 hour prior to the experimental session) to rats performing on a differential-reinforcement-of-low-rate schedule (DRL > 18-sec.) of water rein­forcement increases reinforcements and decreases responses.

Withdrawal from social interactions is one of the striking symptoms of psychosis that has received little experimental attention. The preclinical study of antipsychotic drugs has focused mainly on their ability to block stereotyped motor acts induced by dopaminergic agonists. It was our objective to induce "psychotic-like" changes in social behavior in members of established troops of squirrel monkeys by repeated administration of 6-­methyltyrosine or cocaine, to study the effects of haloperidol, chlorpromazine and physostigmine on psychostimulant-induced suppression of social communication, and to compare these effects to those on motor stereotypes. In the first series of experiments, either d-amphetamine (1 mg/kg) or cocaine (10 mg/kg) were injected i.p. three times within 24 hrs to one of the adult male members of each of four colonies of Saimiri sciureus (n=6-9).

The third injection was preceded by administration of chlorpromazine (0.25, 0.5, 1.0 mg/kg, i.p.), haloperidol (0.25, 0.5 mg/kg, i.p.) or physostigmine (0.04, 0.08 mg/kg, i.m.). At the time of peak effect, an uninformed observer, using the focal animal technique, measured the frequency and duration of all social behavioral ele­ments that the drugged monkey initiated or was recipient of (genital display, displacing, grasping, huddling) via a computer-based keyboard system. In addition, the level of motor activity was assessed and the movements were counted as the most prominent stereotype. We found that d-amphetamine and cocaine se­verely decreased all social interactions and increased stereo­typy when administered alone. Chlorpromazine and haloperidol and physostigmine blocked the cocaine- and amphetamine-induced stereotypes and changes in motor activity. Haloperidol attenuated social with­drawal due to amphetamine, but physostigmine, which avoided side-effects that interfered with social behavior. Our data pro­vide evidence that psychostimulant-induced changes in primate so­cial behavior are highly sensitive to the action of antipsychotic drugs and may be based on different mechanisms than those for motor stereotypes.
INSSENSITIVITY OF THE DORSAL RAPHE TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF LSD. D.J. Minnema*, G. Krymchak* and J. Rosencrans.

Dept. of Pharmacol., Med. Col. of Va., Richmond, VA 23298.

Previous studies have strongly suggested that the dorsal raphe may be a major site of LSD action. To examine this possibility fourteen male Sprague-Dawley rats were trained to discriminate 96 µg/kg LSD from saline on a VI-15 second schedule of reinforcement for sweetened milk using a two lever operant paradigm. Operant sessions were 15 minutes long. The accuracy of LSD discrimination was determined during a 2.5 minute extinction period which was presented every fourth day preceding the training session. Once reliable discrimination was obtained (i.e. > 80% drug-lever responding with LSD, < 20% drug-lever responding with saline), chronic, indwelling guide cannulas for microinjection were stereotaxically implanted into the area of the dorsal raphe. After reestablishment of reliable LSD discrimination, various doses of LSD were administered via a 31 gauge needle into the dorsal raphe. The LSD was dissolved in artificial CSF and delivered in volumes not exceeding 0.5 µl. Extremely high doses of LSD (> 50 µg/kg) administered to the dorsal raphe were needed to produce generalization (> 90%) to the drug lever. A four session study indicated that the duration of LSD generalization of the centrally administered drug closely mimicked the duration of peripherally injected drug. These results suggest that the dorsal raphe is relatively insensitive to the discriminative stimulus properties of LSD when the drug is applied directly at this site. The time-course data suggest that the LSD may be rapidly diffusing away from the site of injection and acting directly, or through the circulation, at other sites in the brain. (Supported by U.S.P.H.S. grant FOA-00296-05).

BEHAVIOURAL SUPERSENSITIVITY TO DopAmINE-MIMETICS AFTER REPEATED AGoNIST OR ANTAGoNIST TREATMENT, INVOLVEMENT OF AUTORECEPTORS. Pavel Muller, Torgny H. Svensson and Arvid Carlsson* Dept. of Pharmacol., University of Goteborg, S-400 33 Goteborg, Sweden.

Long-term treatment with both dopamine agonists and antagonists induces augmented behavioural responses to dopamine mimetics. This phenomenon has been attributed to sensitization of postsynaptic dopamine receptors. However, the postsynaptic receptor changes after long-term neuroleptic treatment develop much later than the behavioural sensitization for which they were proposed to account. Thus, an enhanced response to apomorphine was reported already 24 hours after single injections of neuroleptics. In addition, long-term amphetamine treatment does not appear to produce any postsynaptic receptor supersensitivity. We report here the results of behavioural and electrophysiological experiments designed to study the function of dopamine autoreceptors in rats following single or repeated agonist or antagonist treatment. Our experiments indicate that 24 hours after a single injection of haloperidol (0.5-5 mg/kg) the autoreceptor response to exogenously administered apomorphine is reduced, probably due to blockade of these receptors by residual haloperidol. Long-term amphetamine treatment (5 mg/kg daily for 2 weeks, 48 h withdrawal) induced desensitization of the autoreceptors, while leaving the postsynaptic dopamine receptors apparently unaffected. The blockade of the autoreceptors by haloperidol or their desensitization after chronic amphetamine treatment implies a reduced “presynaptic” inhibition of dopamine neurons by exogenously administered dopamine mimetics. Thus, more dopamine will be available to stimulate the postsynaptic receptors together with the exogenous agonist. Consequently, an augmented behavioural response to exogenously administered dopamine-mimetics such as apomorphine may be the outcome of a reduced dopamine autoreceptor function.

Supported by the Swedish Medical Research Council (grants 155 and 4747) and the Medical Research Council of Canada.
Rensselaer Polytechnic Institute, Troy, NY 12181.

In a number of recent studies it has been shown that the spontaneously hypertensive rat (SHR), a strain derived from the normotensive Wistar Kyoto rat (WKy), is hyperactive as well as hypertensive. Increased locomotor activity in the open field test was seen in both young (4-5 wk) and older (9-13 wk), male and female SHR (Myers et al., Fed. Proc. 36, 1977). We now report that both peripheral and central injections of d-amphetamine decrease locomotor activity in the SHR.

In the first study 10-12 wk old SHR and WKy rats were injected with d-amphetamine (5 mg/kg i.p.), and after 45 min were tested for 5 min in an open field. We found that while the WKy gave the expected increase in locomotor activity, SHR demonstrated a significant reduction in activity. In a second experiment SHR and WKy rats were given systemic d-amphetamine in doses of 1.0, 2.0 or 3.0 mg/kg i.p., and then tested in an automated activity box for 1 h following injection. As in the previous study, WKy increased while SHR decreased their activity in a dose-dependent fashion and at all doses tested.

We sought to localize a central nervous system structure involved in these effects of d-amphetamine. The nucleus accumbens is known to be important in the control of spontaneous locomotor activity and it is known that d-amphetamine injection into this area increases locomotor activity (van Rossum et al., Adv. Biochem. Psychopharmac. 16:201, 1977). Female SHR and WKy rats were bilaterally implanted with cannulae in the nucleus accumbens, and 1 wk later were injected with either saline (1 μl) or d-amphetamine in saline (10 μg in 1 μl), bilaterally in the n. accumbens. Each subject's baseline score from the score in question) between groups with d-amphetamine (5 mg/kg i.p.), and after 45 min were tested for 5 min in an open field. We found that while the WKy gave the expected increase in locomotor activity, SHR demonstrated a significant reduction in activity. In a second experiment SHR and WKy rats were given systemic d-amphetamine in doses of 1.0, 2.0 or 3.0 mg/kg i.p., and then tested in an automated activity box for 1 h following injection. As in the previous study, WKy increased while SHR decreased their activity in a dose-dependent fashion and at all doses tested.

These results suggest the possibility of opioid involvement in ethanol's suppression of female sexual reflexes. The subjects were 24 ovariectomized, estrogen and progesterone-primed female hamsters, randomly assigned to 4 groups. Each group received two injections: (a) ethanol and naloxone, (b) ethanol and saline, (c) saline and naloxone, or (d) saline and saline while measured for 5 days of chronic administration. The threshold elevation was not reversed by naloxone given 0.5 hr after the final morphine treatment. In contrast, acute morphine significantly lowered self-stimulation threshold in either brain locus.

The present data suggest a specificity of action of opiates on different brain systems subserving reward and reinforcement. These findings also support that the mesolimbic-mesocortical system may constitute one focal area mediating the rewarding properties of morphine. (Supported, in part, by USPHS grant DA 01560.)

The contribution of behavioral compensation to tolerance development to physostigmine (0.5 mg/kg once daily) in rats was assessed by comparing the rates of tolerance development to physostigmine's suppressant effects on operant behavior (responding for water reward on an F5 schedule) in groups treated chronically either before or after the operant session. These groups exhibited comparable rates of tolerance development to physostigmine, indicating that behavioral compensation does not play a major role.

Challenges with the muscarinic receptor agonist, pilocarpine, revealed that the operant responding of the physostigmine-tolerant and control groups was suppressed to comparable degrees, suggesting that a decrease in the sensitivity of muscarinic receptors does not play a role in tolerance development to physostigmine. Binding assays with the specific muscarinic antagonist, 3H-dzemide, revealed similar densities of muscarinic binding sites in the tolerant and control groups, suggesting that a change in muscarinic receptor properties is also not involved in tolerance development to physostigmine. These findings suggest that behavioral compensation and alterations in muscarinic receptors are unlikely mechanisms underlying tolerance to physostigmine. Therefore, mechanisms underlying tolerance to carbamate anticholinesterases appear to be different from those underlying tolerance to organophosphates (see Schiller, Life Sciences 24, 1979). (Supported by a grant from the Australian Research Grants Committee to D.R. Overstreet and USPHS grants to N.I. Yarmemra.)
CONCENTRATION RATIO

0.84±0.05
0.90±0.03
0.72±0.03
0.96±0.00
0.77±0.02

Ethanol Alone


We studied imipramine and several of its analogs for their ability to inhibit muscarinic receptor-mediated cyclic GMP (cGMP) synthesis by mouse neuroblastoma cells. The rank order of potency of these drugs as antagonists for imipramine and its analogs: 1) The type of amine on the side chain is predictive of antimuscarinic activity with tertiary > secondary > primary; 2) 2-hydroxyl ring substitution was more effective than 3-hydroxyl; 3) the 7,8-diOH-CPZs were more potent than the 7,9-diOH-CPZs; 4) the 3-chloro-2-hydroxy imipramine was more potent than the 3-chloro-2,3-dihydro imipramine; 5) 7,8-diOH-CPZs were more potent than 7,9-diOH-CPZs and the latter inhibited the basal enzyme activity; 6) the 7,8-diOH-CPZs were more potent than the 7,9-diOH-CPZs.

The results are as follows. The absolute plasma ethanol concentration constant and the inhibition constant (K_i) was calculated assuming competitive inhibition at the muscarinic receptor and using a K_m = 200 μM for carbamylcholine (E. B. Rushing, unpublished data). The K_i was essentially identical to the equilibrium dissociation constant (K_d) as determined by the dose-ratio method for the compounds which were tested by both techniques. The muscarinic antagonists as follows was for: imipramine = 3-chloroimipramine > desmethylimipramine > 3-chloro-2-hydroxy imipramine > 2-hydroxyimipramine > dixemethoxyimipramine. The most potent drugs, imipramine and 3-chloroimipramine, were about 30 times more potent than the least potent compound, dixemethoxyimipramine. These results suggest that the following structure-activity relationships for 3-chloroimipramine and its analogs: 1) The type of amine on the side chain is predictive of antimuscarinic activity with tertiary > secondary > primary; 2) 2-hydroxyl ring substitutions markedly reduce activity of these compounds; and 3) 3-chloro and N-oxide substitutions have no effect on antimuscarinic activity.

Thus, AMI increased not only the diffusion of alcohol across the cerebral capillaries but also results in a net influx of alcohol into the brain. This new observation has both clinical and basic neuroscience implications with regard to understanding drug interactions such as TCA and ethanol.
PSYCHOPHARMACOLOGY


Monkeys (Macaca fascicularis) dosed orally with 500 mg/kg/day of lead acetate showed deficits compared to controls on a series of 20 form discrimination reversals at 2-3 years of age. There was no difference compared between groups in the effect of 'overtraining' trial introduced between reversals. Treated monkeys also showed a decreased ability to control initial sessions of a multiple fixed interval (FI) 8 min. - time out schedule of reinforcement, with lead exposure producing increased FI response rates, a "bursting" pattern of FI responding, and increased time out responding. Subtle differences in the pattern of FI responding persisted throughout the course of the study as evidenced by differences in inter-response time distribution. Blood lead levels at the time of behavioral testing were 20-40 ug/dl, which are comparable to lead levels of many children.


The effect of bilateral infusions of 6-hydroxydopamine (6-OHDA) into the n.accumbens on measures of cocaine reinforcement: intravenous self-administration and facilitation of intracranial self-stimulation (ICSS).

Male Wistar rats were prepared with intravenous cannulae and trained to self administer cocaine at a dose of 0.75 mg/kg/inj. Injections were delivered with each depression of a lever during a daily 3 hr session. After self-injection of cocaine had stabilized each rat received bilateral infusions of 6-OHDA into the n.accumbens. All rats (N=15) showed an initial abstinence from cocaine self-administration. However, many rats showed a gradual recovery of cocaine intake. The number of post lesion days required to reach 50% recovery of pre-lesion cocaine intake correlated (r=0.41) with dopamine (DA) content remaining in the n.accumbens. The same rats which failed to respond for cocaine continued to self administer amphetamine (0.6 mg/kg/inj.) at pre lesion levels.

In an effort to establish if these lesions would also block the facilitation of ICSS from cocaine, 16 rats were prepared with electrodes in the posterior hypothalamus. Animals were allowed to press a lever for intracranial electrical stimulation and were tested for 5 min at eight current levels in a descending series. Half of the animals received bilateral injection of 6-OHDA (8ug/2ul) into the n.accumbens while the remaining half received vehicle injections (saline containing ascobic acid, 0.1% v/v). Twelve days following lesion the animals were injected with cocaine (10mg/kg) 15 min prior to the test session. In the control animals, cocaine produced a significant shift to the left of the rats' dose function. By contrast, the 6-OHDA group showed an attenuation of this cocaine facilitation of ICSS. These results add further support to the hypothesis that mesolimbic dopamine serves a critical role in cocaine reinforcement.


Animals are known to exhibit sensitization with repeated amphetamine (AM) administration: in particular, single doses of AM can cause enhanced stereotyped behavior to a subsequent dose several days later. In man, AM psychosis closely resembles some forms of schizophrenia, a disease whose etiology is a combination of genetic factors and environmental stress.

In the first set of experiments, we have investigated whether stress and AM may act upon the same neural substrate(s) involved in sensitization in rats. We have already shown (Neurosci. Abstr. #154, 1978) that repeated application of a mild stress, tail pressure (TP) produces an enhanced stereotyped response to a single dose of AM. We now report the converse experiment: a single dose of AM can produce a long-lasting "sensitization" of TP-induced eating and grooming. This is, when tested up to 30 days after the AM injection, rats show a smaller haloperidol (HALO; 0.4 mg/kg)-induced suppression of TP behaviors than do vehicle injected controls. Similarly, a single session of footshock stress produced an enhanced AM stereotypy response two weeks later and a decreased efficacy of HALO in the TP test. To identify brain structures which might be associated with sensitization, we examined the effect of another stressor, chronic self-stimulation, which offers the advantage of site specificity, on the ability of AM later to induce stereotyped sniffing and anorexia. Nucleus accumbens and medial frontal cortex self-stimulators (but not rats with A-9 placements) showed a significant enhancement of each of these effects of AM when tested 24 hr after the last stimulation day. Since we have previously reported that food deprivation stress can produce similar sensitization, it appears that a wide variety of stresses can induce the effect.

In a second experimental series we have investigated the behavioral effects of AM in spontaneously hypertensive rats (SHR) which are known to show enhanced behavioral and adrenomedullary responses to stress and therefore may be thought of as a genetic or naturally stressed preparation. We report that single doses of AM produce more intense and prolonged stereotyped behavior in SHR rats than in either WKY or Sprague-Dawley (SD) controls. HALO was less effective in blocking TP behavior in SHR's than in controls, but was more effective in inhibiting these animals. This last finding suggests that the level of activation of the testing situation is an important variable in assessing the reactivity of brain catecholaminergic systems.

2254 EFFECTS OF CHRONIC ADMINISTRATION OF MORPHEINE ON THRESHOLDS FOR ESCAPE BEHAVIOR MAINTAINED BY INTRACRANIAL STIMULATION. Stephen Sasson*, Howard S. Wheeling*, and Conan Kometsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA. 02118

It has been previously demonstrated that acute doses of morphine lower the threshold for intracranial self-stimulation (ICSS) and raise the threshold for escape from stimulation to 'aversive' areas of the brain (Marcus & Kometsky, Psychopharmac., 38:1, 1974). Work in our laboratory has also shown that tolerance does not develop to the threshold lowering effect of morphine on ICSS (Esposito & Kometsky, Science, 195:1897, 1977). The present study is an attempt to determine whether or not tolerance develops to the threshold raising effect of morphine for escape from aversive brain stimulation. Six male albino rats were implanted with bipolar electrodes aimed at the mesencephalic reticular formation (RF). They were trained to self-administer a wheel manipulandum in order to escape RF stimulation. Thresholds were determined by means of a modification of the psychophysical method of limits. After training and prior to the initiation of daily morphine injections, data were collected for at least 5 days in which saline was administered. Animals were given daily doses of morphine (4-20 mg/kg, s.c.) for 14 to 40 days. Starting with the first day and every 2 to 3 days thereafter, thresholds were determined before and 25 min after a test dose of morphine. The dose of morphine selected as the test dose initially raised the threshold at pre-lesion levels to 50-100% of the pre-lesion saline level for the respective animal. Results indicate that tolerance does develop to the threshold raising effect of morphine; however, this loss of threshold raising effect is often complicated by the disruption of behavior caused by abstinence. These results show that the phenomenon of tolerance to the antinociceptive effect of morphine is observable whether administered peripherally or centrally applied noxious stimuli are utilized. (Supported by NIDA Grant DA 0037, Biomedical Research Support Grant at Boston University School of Medicine, and Research Scientist Awarded NR 1759 - CX)
H-5HT SEROTONIN RECEPTOR BINDING IN RAT BRAIN: DIFFERENTIAL EFFECTS OF ANTAGONISTS AND SEROTONIN RE-UPTAKE INHIBITORS


While the administration of antipodaline (10mg/kg, bid, for 16 days) caused no significant alteration in either the concentration of serotonin or the binding of H-5HT in the brain regions examined, injection of the monoamine oxidase inhibitor transylcypromine (3mg/kg/day) for an equivalent period of time caused a significant reduction in H-5HT binding, both in cerebral cortex and pons.

At no time up to 16 days of administration did either of the serotonin uptake inhibitors fluoxetine (10mg/kg, bid) or cloripramine (10mg/kg, bid) change H-5HT binding in comparison with that measured in control rats. In contrast, a significant reduction in H-5HT binding and an increase in the concentration of serotonin was measured in cerebral cortex and pons 24 hours after single injection of the monoamine oxidase inhibitor nialamide (40mg/kg). This effect persisted up to 16 days of treatment with nialamide.

Treatment with the relatively selective A type monoamine oxidase inhibitor clorglyine (1.0mg/kg/day, for 4 days) resulted in a significant increase in brain serotonin and a reduction in H-5HT binding, while treatment with the B type inhibitor (1.0mg/kg/day, for 4 days) or pargyline (1.0mg/kg/day, for 4 days) resulted in no change either in binding or brain serotonin concentrations.

Analysis by the method of Scatchard of the changes seen in cerebral cortex indicated for four days a marked increase in the apparent binding constant (Kd), with no change in the maximum specific binding capacity (Bmax).

In conclusion, chronic treatment of rats with serotonin reuptake inhibitors has effect on the H-5HT system, while the monoamine oxidase inhibitors caused significant elevations in brain serotonin levels and decreased H-5HT binding. (Supported by Research Funds from the Veterans Administration, 900 Grant 29094, and USPHS GM 07302).

DIFFERENTIAL EFFECTS OF ANTIANXIETY AND ANTIPSYCHOTIC DRUGS ON TWO DISTINCT SUBSYSTEMS OF AROUSAL


The behavioral signs of social withdrawal were induced in rats by administration of 5-MeODMT (1.25 mg/kg) or saline injection. The behavioral signs of social withdrawal were measured using a cross-over design so that each monkey in the colony received each treatment at least once. The results showed that administration of 5-MeODMT produced a marked disruption of behavior which helped us interpret the relation between chemical and clinical potency.

5-MeODMT is a hallucinogenic substance which has been postulated as an endogenous psychotogen in humans. We have previously reported that 5-MeODMT induces abnormal behavior and significantly alters normal affiliative behavior when administered to selected members of primate social colonies. (Comm. Psychopharmacol. 1:105, 1977). The present studies were designed to examine the role of serotonin systems in the mediation of social withdrawal in rats. All experiments were performed using stable social colonies of five to six adult stump-tailed macaques. Each experiment began with a baseline observation period followed by administration of 5-MeODMT. The animals were given saline injections with 5-MeODMT and saline injections with 5-MeODMT.

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2262 EFFECT OF SOME AMPHETAMINE ANALOGUES IN RATS TRAINED TO DISCRIMINATE BETWEEN L-DOPA AND SALINE. Peter B. Silverman and Jing T. Ho. Div. of Psychiatry, University of Texas, Houston, TX 77030.

We have previously demonstrated that chronic haloperidol treatment (21 days at 1 mg/kg/day) causes an increase in intracranial self-stimulation (ICSS) rate in rats implanted with electrodes in the ventral tegmental nucleus. This increase persists for three weeks at full magnitude, before declining to baseline rates at four weeks after the termination of haloperidol treatment. This long-lasting period of supersensitivity allows for the testing of pharmacological agents which may modify or reverse the neuroleptic-induced increase in ICSS rate. In the first experiment, 31 rats which demonstrated ICSS at high rates were given three consecutive tests to establish a baseline rate. Of these rats, 16 of the rats were then given three weeks of haloperidol injections. The rats were retested at 3, 5 and 7 days after haloperidol withdrawal, and at this time demonstrated an average 50% increase in ICSS rate. After the test on day 7, 8 of these rats were given 100 mg/kg L-DOPA plus 20 mg/kg Carbiparid daily for the next seven days. These rats showed a progressive decline in ICSS rate, leveling off at 120% of baseline rate, and persisting at this level for one week after the last L-DOPA treatment. The 8 rats which received saline injections were given three consecutive tests to establish a baseline rate. Five rats given L-DOPA and Carbiparid for one week without previous haloperidol injections were then given three weeks of haloperidol injections during or after the L-DOPA treatment. 10 control animals given saline injections for three weeks also showed no significant changes. These results indicate that chronic L-DOPA treatment causes a reversal of neuroleptic-induced behavioral supersensitivity, at a dosage level which does not by itself cause subsensitivity (i.e. a decrease in ICSS rate). In the second experiment, 8 rats were given daily lithium carbonate in their food (1600 mg/kg of powdered chow) concurrently with three weeks of haloperidol injections (1 mg/kg/day). In this group, a significant but significant increase in ICSS rate was seen at 3 and 5 days after the last haloperidol injection, with the rate declining to baseline levels at day 7. This result suggests that lithium treatment attenuates both the magnitude and time course of neuroleptic-induced supersensitivity. The current results demonstrate the reversal of supersensitivity in a behavior mediated by the mesolimbic dopamine system, and agree with the findings of other investigators who have used behaviors mediated by the nigro-striatal dopamine system. They suggest the possible usefulness of pharmacological desensitization in disease states which may involve abnormal receptor sensitivity, such as tardive dyskinesia and schizophrenia. (Supported, in part, by USPHS research grant DA-02089.)
Chlorpromazine (CPZ) has been reported to decrease or abolish avoidance responses (ARs) and escape responses (ERs) in rats trapped in a shuttle box. Closer analysis reveals that rigorous time constraints placed on rats trapped to release a bar from a lever (one basic requirement: escape responses (ERs) to the unconditioned stimulus (2.5 mA shock), Avoidance Responses (ARs) to a conditioned stimulus (light and buzzer), and Orienting Responses (ORs) to both the conditioned and unconditioned stimulii). In a study of effects of CPZ, ER, OR, and AR were analyzed using a highly trained response approaching the animal's speed capacity.

Boltzman rats (15) were trained over a 6 day period to depress and hold a lever and to release it when the auditory/visual stimulus was presented. Minimal latency responses were redefined by reducing the CS-UCS interval in steps of 100 sec. Following training, animals were pretested over 50 trials decreasing intervals, then were injected with CPZ (Thiorazine, 3.5 mg/kg). After 40 minutes the animals were tested, ER and AR latencies were determined by bar release, while the latency of the movement initiation was detected by high speed cinematographic analysis. Film analysis also provided characteristics of the movement patterns for ER, AR, and OR.

Avoidance responses increased during training to 60-10% occurrences by the 6th day for all rats. CPZ produced increased decrements in percent avoidance with decreased CS-US intervals, and at the 200 sec interval only 4 animals were capable of avoiding. Contrasting results were obtained for the response delay escape responses. CPZ avoidance latencies were slower only at the least constrained CS-US interval of 1000 msec and at the shortest interval for the most animals capable of responding.

The most striking of the qualitative differences between ERs and ARs was that the initial component of the ER was a total body jump, while the initial component of the AR was a characteristic movement of the head and/or forelimbs. A major effect of CPZ on the movement pattern was a change in starting position, which may have been related to the CPZ-induced loss of nausea in the AR with a postural adjustment. Finally, film analysis confirmed that CPZ delayed the onset of the AR and expanded the temporal aspects of the movement pattern.

These results show that when escape and avoidance behaviors (a) are initiated at speed capacity, (b) start from a learned and maintained baseline, and (c) require rapid cinematographic analysis, CPZ produces results that deviate from those previously reported. (Supported by University Research Institute, UT Austin)

Older age is associated with changes in catecholamine neurons and neurotransmitters and their receptors. Tardive dyskinesia, a long-term toxic effect seen after chronic administration of neuroleptics to man is much more prevalent in older-age humans. To investigate whether receptor changes may be involved in these phenomena, we are studying the effects of age and chronic neuroleptics on receptor binding in rat brain. Fisher 344 rats, treated chronically with fluphenazine or sal­ine, sacrificed 9-10 after termination of chronic injections, were used in all experiments. Receptor binding for dopamine, adrenergic, and cholinergic receptors was assayed using tritiated spiperone, DHA, WR-4101, DHE, and QNB using established techniques. Our preliminary results show a significant decrease in specific binding of DHA, and WR-4101 in the cortex of old rats, a significant decrease in spiperone binding in the striatum of old age rats. Kinetic analysis showed a decrease in Bmax but no change in k in the spiperone binding in the striatum of old rats. Specific binding of DHE in the cortex and QNB in the hippocampus showed a curvilinear pattern in relation to age. Prior treatment with chronic neuroleptics slightly increased DHA binding in the cortex of young rats, but significantly decreased DHA binding in the cortex of old rats. Further kinetic studies of adrenergic and dopaminergic binding, and the interaction of age and chronic neuroleptics on dopaminergic vs dopaminergic binding are currently being pursued.

Bupropion HCl, a non-tricyclic compound with antidepressant properties in man, was evaluated for effects on plasma prolactin (PRL) and growth hormone (GH) levels in normal human subjects, and for effects on plasma PRL levels in a series of pharmacological studies in normal rats. Single oral doses of 50 or 200 mg of bupropion given to 6 male and 12 female normal volunteers produced a marked suppression (up to 70% decrease) of plasma PRL levels for at least 4 hours (see Table). At the end of 24 hours plasma PRL levels were still below normal values. At one hour after drug administration there was a +.56 correlation (p <.05) between bupropion plasma levels and the percentage decrease in PRL levels. On the other hand, GH only showed small and erratic changes in plasma levels. In the rat, single doses of 10 or 25 mg/kg, i.p., of bupropion failed to lower PRL levels. However, bupropion at 25 mg/kg, i.p., significantly decreased PRL in rats in which baseline PRL levels were elevated by pretreatment with alpha-methyltyrosine (AMT at 150 mg/kg, i.p.), 5-hydroxytryptophan (5-HPT at 100 mg/kg) or quipazine (10 mg/kg; see Table).

Overall, the results in man and rat are consistent with the view that bupropion has significant dopamine agonistic properties. Whether bupropion is a directly or indirectly acting dopaminergic agonist cannot be determined from the present results.


Drugs of abuse have been shown to modulate mechanisms of reward through their action on catecholaminergic systems. Following lesioning of the locus coeruleus (LC), rats were run for 21 days of saline in a self-stimulation paradigm. ICSS drug paradigms post-lesion were identical to the pre-lesion paradigm. It was found that some ICSS sites which had in common that they were both equally potentiated by the d- and l-isomers of amphetamine and also showed a facilitation under morphine. In contrast, sites which showed a greater facilitation under morphine, maintained their facilitation under morphine after the LC lesion, whereas sites which showed a facilitation under morphine, maintained their facilitation, which was naloxone reversible, after the LC lesion. Relevance of this data to the effects that opiate centers modulate catecholaminergic effects on ICSS will be discussed.

AGONIST POTENCIES AT THE DOPAMINE RECEPTORS. M. Titeler and E. Seeman. Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

A series of ergot derivatives have been assayed for their ability to compete for the specific binding, in calf caudate homogenates, of [3H]-spiperone, a dopamine receptor antagonist (1), [3H]-dihydroergocryptine (3H-MECA) a dopamine receptor agonist (2,3), and [3H]-dopamine, the neurotransmitter. Bromocryptine has been shown to be a potent dopamine receptor agonist in vivo (2) and in vitro (3), and this drug demonstrates a 10-20 fold higher affinity for the [3H]-spiperone and [3H]-dihydroergocryptine specific binding sites than for the [3H]-dopamine binding site. Dihydroergocryptine has also been shown to be a potent dopamine receptor agonist in vivo (4) and in vitro (5), and this drug demonstrates a 10-20 fold higher affinity for [3H]-spiperone and [3H]-MECA specific binding sites than for [3H]-dopamine sites. These data along with other data on location and function of these sites indicate that 3H-MECA and [3H]-spiperone label the same site, the post-synaptic dopamine receptor, and that [3H]-spiperone labels another dopamine neuronal structure, possibly the dopamine autoreceptor (4). These data cannot possibly be interpreted in terms of the two-state model of the dopamine receptor, as has been suggested (5).
ACUTE AND CHRONIC TOLERANCE TO A BEHAVIORAL EFFECT OF NICOTINE IN RATS. Glenn Daniel Todd* and John A. Dougherty* (SPON: Ralph E. Miller). VA Medical Center, Dept. Pharmacology and Psychiatry, Univ. of Kentucky, Lexington, Ky. 40507.

Acute and chronic tolerance to the behavioral suppressant effects of nicotine was examined in rats that had previously been trained by water-reinforced lever pressing according to a fixed-ratio 50 schedule. The rats were maintained at 80% of their free-feeding weight by giving supplemental water access after the second daily session. The overall response rate after saline pretreatment was used as a predrug baseline with which to compare the effects of nicotine dosing.

The first nicotine injection (200 μg/kg, BASE) decreased overall response rate by about 50% in all four groups. However, the second nicotine injection, 1.25, 4, or 8 hours later on the same day had no significant behavioral effect. On the second day this pattern was repeated, with nicotine again decreasing the responding after the first, but not the second injection. Over nine consecutive days, the response rates after the first daily drug injection gradually recovered to within predrug ranges, while the behavior after the second injection remained unchanged or slightly stimulated when compared to controls. There were no differences between groups in drug effects on responding.

After the initial pause, responding was abruptly resumed at the predrug rate. This pause was unrelated to motor impairment. The pause gradually decreased in duration over the nine day period and was not apparent after any of the second daily injections.

A subsequent dose increase to 350 μg/kg (BASE) twice a day reinstated the initial suppression with the development of tolerance over nine days. The pattern seen with the lower dose of nicotine was repeated.

These results indicate that a rapid tolerance occurs to the suppressant effects of nicotine within one hour and persists up to eight hours, but not for 16 to 23 hours. A more gradual tolerance also occurs after 6 to 9 days of injections. This biphasic development of tolerance suggests that more than one process may be involved.


Pigeons were trained to discriminate an intramuscular injection of naloxone (NTX; 32 or 56 μg/kg) from saline by reinforcement of responses on one of two keys. Following NTX administration, 20 consecutive responses on the appropriate key resulted in 4 sec access to grain, while responses on the other key were reinforced when saline was administered. During test sessions, 20 consecutive responses on either key resulted in grain delivery. Naloxone generally caused both periods of no responding to be evident and NTX. Administration of behaviorally active doses of cyclazocine, amphetamine, and ethylketazocine did not result in drug-appropriate responding. Quaternary NTX produced appropriate responding in 3 of 5 out of 3 out of 5 birds. One bird generalized completely to quaternary NTX, while another responded only partially on the drug-appropriate key. NTX-10, 100, and 15 μg/kg (1, 2, and 3; hydroxy-6-methyl-3-benzoazaine) produces a syndrome resembling the narcotic abstinence syndrome in narcotic-naive monkeys. In the isolated guinea-pig ileum preparation, UM-1046 produces a contract

The administration of UM-1046 to mirtrexone-trained pigeons produced complete NTX-appropriate responding in 2 of the animals and partial generalization in two others. UM-979 ((-)-5, 9-dimethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorph), a drug with narcotic-antagonist properties, produced results similar to the administration of UM-1046. These data indicate that mirtrexone has discriminative effects in nondependent animals which may be distinguished from those of other narcotic agonists and mixed narcotic agonist-antagonists. Characterization of narcotic antagonists using drug discrimination procedures may be useful in determining the effects of these drugs in both the naive and narcotic-dependent state. (Supported by NIDA grants DA 00154 and 00154.)


PCP effects can be inhibited by haloperidol. These data strongly suggest that dopamine(DA) is involved in the mediation of these effects. The purpose of this study is to examine the effects of PCP on tyrosine hydroxylase(TH), the rate-limiting enzyme in the synthesis of DA. A crude synaptosomal homogenate was prepared by homogenizing pooled striata from untreated male Sprague-Dawley rats in 20 vol. of 0.32 M sucrose. The synaptosomal tissue was collected by centrifugation and resuspended in 20 vol. of either 37 C or ice-cold physiological phosphate buffer(pH 6.8) containing β-benzylhydroxyamine(334 μM), an inhibitor of DOPA decarboxylase. Either saline or PCP was added and preincubated for 20 min. The reaction was initiated by the addition of tyrosine(20 μM) containing 1 uCi L-(3,5-3 H)-tyrosine and was stopped after 20 min. by the addition of glacial acetic acid. Tryptophan labelled water, which was formed following hydroxylation of L-(3,5-3 H)-tyrosine by TH, was separated from tryptophan labelled tyrosine and its metabolites by ion-exchange chromatography and identified by liquid scintillation spectrophotometry. TH activity assayed in this manner was completely inhibited by 3-iodotyrosine(2 μM). PCP was obtained by each dosing interval over the range of 0.1 μM to 100 μM with maximal stimulation occurring at 10 μM. On the other hand, PCP had no effect on the turnover of TH-tyrosine at these concentrations. However, little or no stimulation of TH activity was observed at any PCP concentration tested when the synaptosomal preparation was allowed to be incubated for 20 min. at 37 C. Suggesting that PCP may be acting indirectly via the release of DA which normally inhibits TH. These effects are similar to those previously reported for d-amphetamine on synaptosomal TH activity.
Production of Physical Dependence in the Alcohol-Preferring and -Nonpreferring Lines of Rat. Marshall B. Waller, William J. Indianapolis, IN 46223.

This study examines the effect of caloric restriction and flavor additives in the EtOH solution, water for 13 weeks. Ethanol intake remained constant in 3 animals while 5 exhibited a progressive rise in alcohol consumption of withdrawal appeared in 6 of the animals on the first day. Disrupted behavior was exhibited by at least 5 of 8 animals in the first experiment, both P and NP rats were weight-reduced to 80% of their free-feeding weight and given free-choice between water and a 10% EtOH solution flavored with saccharin, 0.125 g%, and NaCl, 1 g%. EtOH consumption increased from 7 to 14 g/kg/d in the P rats and from 1 to 12 g/kg/d in the NP rats. After 8 weeks, alcohol was discontinued and the animals were tested for withdrawal. Physical signs, e.g., tremor, wet dog shakes, teeth chattering, Straub tail, were observed in 8 of 9 rats of the P-Line. Sound from a bell (100 db) induced seizures in 3 animals while 2 others exhibited hyperreactivity to this stimulus. Physical signs were seen in all NP animals. The bell induced seizure in one animal and two showed increased sensitivity to the sound for up to 72 hours post-withdrawal. These signs appeared within the first day. Subsequently, behavioral impairment in the runway test appeared in 7 of 9 P animals and 7 of 8 NP animals tested. Results were similar from a runway test at 24-72 hours post-withdrawal in 7 of 9 P rats and 6 of 8 NP animals tested. In the open field, one P animal exhibited hyporeactivity while 7 animals became hyperactive in this test, 3 NP rats were hyporeactive and only one was hyperactive in this test. In a second experiment, 8 rats of the P-Line were given food ad libitum and the free-choice drinking of unfavored 10% EtOH and water for 13 weeks. Ethanol intake remained constant in 3 animals while 5 exhibited a progressive rise in alcohol consumption from 5 to 9 g/kg/d. When alcohol was discontinued, physical signs of withdrawal appeared in 6 of the animals on the first day. Disrupted behavior was exhibited by at least 5 of 8 animals in the first experiment, head-poke and rearing activities, or the runway test. These data indicate that chronic EtOH consumption, with or without food restriction and flavor additives in the EtOH solution, can produce physical dependence in these selectively bred, alcohol-preferring lines of rats. (Supported by USPHS Grant no. AM003243).

A Behavioral Model of Early Encephalopathy in the End-to-Side Portacaval Shunt Rat. John R. Warbritton, Ill, Mark A. Geyer, Brent Jepson* and Joseph E. Fischer*. Dept. of Psychiatry, Univ. Calif. San Diego, La Jolla, CA, Dept. of Surgery, Harvard Medical School, Boston, MA, and Dept. of Surgery, Univ. Cincinnati Medical Center, Cincinnati, OH 45267.

Although models of hepatic failure in large and small animals abound, models of early hepatic encephalopathy (HE) are difficult to create. A rat with an end-to-side portacaval shunt (PCS) is an extensively used experimental animal for characterizing models of biochemical, histological and functional changes occurring in the liver, but few behavioral abnormalities have been detected. Biochemical changes observed in rats after PCS include increased plasma and brain levels of tryptophan, with increased brain serotonin and its precursor tryptophan, with increased brain serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Accumulating evidence indicates that serotonin may be involved in the modulation of the arterial response in the rat. In the following study, the magnitude of arterial responses to both tactile and auditory stimuli were shown to be abnormally decreased in chronic PCS rats and suggest that such studies may be used to advantage in the study of HE. End-to-side PCS were carried out in groups of 20 rats by a non-suture technique. Rats were studied at weekly intervals 4 and 5 weeks following PCS utilizing a tactile stimulus, an air puff administered through a solenoid-activated valve, and auditory stimulus consisting of 110 dB tones. The results suggest that animals with an end-to-side portacaval shunt exhibit decreased reactivity to both tactile and auditory stimuli. The simplicity of these tests, when carried out under experimental circumstances, suggest that they may be used to assess the effects of pharmacological manipulation of PCS rats in the treatment of hepatic encephalopathy.

Brain Serotonin, Body Weight and Isolation-Induced Aggression in Mice on a Tryptophan-Free Diet. James E. Walters, Marie Larvoy* and Robert Poujoulat*. Biology Department, William Paterson College of New Jersey, Wayne, NJ 07470.

Diets lacking the essential amino acid, L-tryptophan, significantly decrease brain serotonin (5-HT) levels in rats and have been shown to influence pain sensitivity, acoustic startle, sexual behavior and mouse killing. No previous studies of the effects of a tryptophan-free (TF) diet on aggression in mice have been reported. We report here that mouse behavior toward isolated males was increased by prolonged isolation. Isolation-induced aggression can be decreased by repeated injections of fluoxetine or p-chlorophenylalanine or by administering 5-HT receptor blockers. The present study determined the effects of a TF diet on isolation-induced aggression in two strains of mice. Forty Swiss-Webster (SW) and 58 AJL/J mice were isolated in plastic cages. Each mouse had powdered lab chow and water freely available. Two weeks later half of the mice from each strain were randomly switched to a powdered TF or tryptophan control (TC) diet. After 12 days, pairs of mice from the same strain and diet condition were placed in single divided cages for two more days. The divider and food cup were then removed for a 15-minute test period during which latency to fight, flight duration, and head duration were measured. Mice from five pairs of mice in each strain and diet condition were removed after testing and assayed for brain 5-HT concentration. The TF diet significantly decreased brain 5-HT levels in both SW (34%) and SJL (49%) mice. The TF diet produced a 37% decrease in mean body weight for SJL mice after two weeks, and a 22% decrease for SW mice. The TC diet reduced SJL body weight by only 3% and SW by 10% during the experiment. No significant body weights were found for Diet (p><.01), Strain (p><.01) and Diet X Strain interaction (p><.05). The degree of brain 5-HT depletions, there were no significant effects of diet on any of the behavioral measures for either strain of mice. This study shows that a TF diet depletes brain 5-HT in mice but also produces considerable weight loss over a two week period. Strain differences in acceptance of the diet were evident from the differential weight losses. The degree of brain 5-HT depletion which resulted from eating the TF diet may not have been sufficient to reduce isolation-induced aggression. Such was the case for acoustic startle in rats which could only be affected by tube feeding the TF diet (Walters, Davis & Sheard, Psychopharmacology, 1979, in press). This study was supported by a William Paterson College Summer Research Fellowship.


Repeated single daily injections of d-amphetamine in the rat have been reported previously to produce a progressive augmentation in stereotypy and locomotion (Segal and Haddad, 1974; Segal, 1975). However, since amphetamine-induced psychosis in humans is typically associated with shorter intervals between successive amphetamine administrations, we have conducted studies by characterizing the progressive behavioral changes associated with multiple daily amphetamine injections.

Male Wistar rats received a single i.p. injection of the serotonin (5-HT) precursor tryptophan, with increased brain serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Accumulating evidence indicates that serotonin may be involved in the modulation of the arterial response in the rat. In the following study, the magnitude of arterial responses to both tactile and auditory stimuli were shown to be abnormally decreased in chronic PCS rats and suggest that such studies may be used to advantage in the study of HE. End-to-side PCS were carried out in groups of 20 rats by a non-suture technique. Rats were studied at weekly intervals 4 and 5 weeks following PCS utilizing a tactile stimulus, an air puff administered through a solenoid-activated valve, and auditory stimulus consisting of 110 dB tones. The results suggest that animals with an end-to-side portacaval shunt exhibit decreased reactivity to both tactile and auditory stimuli. The simplicity of these tests, when carried out under experimental circumstances, suggest that they may be used to assess the effects of pharmacological manipulation of PCS rats in the treatment of hepatic encephalopathy.


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Learning theory can explain drug tolerance by proposing compensatory skill-learning as the mechanism underlying behavioral tolerance to drugs, i.e., behavioral tolerance is the result of learning to compensate behaviorally for drug-induced impairment of behavior. This suggests that animals given the opportunity to learn to cope with other, non-pharmacologically-induced but otherwise similar, impairments of behavior would be tolerant to drug-induced behavioral impairment. The present experiment tested and confirmed this prediction. Drug-naive rats, previously trained to walk on a treadmill in order to avoid footshock, were spun daily for 3 min at 120 rpm to render them dizzy and then immediately made to walk on the treadmill. Their performance improved over a 10-day treatment period suggesting that they were becoming tolerant to the effects of the rotation treatment. They were then tested on the treadmill after an injection of diazepam (9 mg/kg ip), an ordinarily disabling dose, and found to be behaviorally tolerant relative to appropriate control animals. Since this was the first time these animals had ever received drugs, classical mechanisms of tolerance were eliminated as potential explanations. Hence, these data support the learning theory interpretation of behavioral tolerance to drugs.

In a two-lever, water-reinforced drug discrimination task male albino rats (n = 15) were trained to discriminate 1.0 mg/kg of fenfluramine (FF) from saline. Within 16 sessions all animals reached a criterion of at least 85% correct responding. Generalization (transfer) testing, conducted during extinction, revealed an orderly increase in per cent FF responding following doses of .25, .50 and .75 mg/kg FF. Three serotonin agonists, quipazine (QP), LSD and MK 212 were also administered during transfer tests. Each of these compounds showed dose-related generalization to the FF cue: responding on the FF-appropriate lever reached 82% following a dose of 2.0 mg/kg of QP; 66% FF responding occurred following .16 mg/kg of LSD and 88% FF responding was observed after 1.0 mg/kg of MK 212. Thus, at the doses tested, the order of transfer potency was MK 212 > QP > LSD. These results confirm reports that FF exerts its behavioral effects primarily by interacting with the serotonergic neuronal system, although this interaction may be indirect (Ann. NY Acad. Sci. 305:222, 1978).

Stereoselectivity for the (-) isomers of DOM and of its cyclopropyl analog in eliciting either mouse ear scratching or cat lamb flicks was observed in our earlier study in which single doses of the compounds were tested (NIDA Research Monograph 22: 70-81, 1978). This study was aimed at further evaluating mouse ear scratching as an animal model for predicting hallucinogenic activity. Doses calculated for eliciting 100 scratching episodes in 30 min were: 0.7 mg/kg for (-) DOM; 1.2 g/kg for (+) DOM; 7.0 mg/kg for escalin and 24 mg/kg for mescaline. Head twitches, but no ear scratches were observed with either (+) DOM or quipazine. Thus the mouse ear scratch response was stereoselective and ranked the phenethylamine-type compounds in order of observed human potency. Phencyclidine induced a low level of scratching activity. Both LSD (0.1-125 ug/kg) and psilocybin (1.5 and 6 mg/kg) failed to elicit the scratching response. Though not sensitive to tryptamine-type compounds, the mouse ear scratch response may be an inexpensive and convenient initial test for predicting potential hallucinogenic activity of phenethylamines. (Supported in part by USPHS Grant DA0191601 and a Purdue Research Foundation Fellowship (to W.R.P.).


Long-term amphetamine treatment results in tolerance to some drug-induced behaviors, (e.g., anorexia), whereas other drug-induced behaviors do not undergo tolerance (e.g., stereotypy). In fact, following chronic exposure to amphetamine, drug-induced stereotypes are exacerbated. Although the enhanced stereotypes following chronic amphetamine treatment may involve several factors, it appears that the increased neuronal efficacy of dopamine (DA) transmission induced by chronic exposure to drug plays a primary role in this respect. In the present study we examined the effects of chronic amphetamine treatment on intracranial self-stimulation (ICSS) from the substantia nigra in order to determine whether chronic drug treatment would modify ICSS and whether these changes parallel those observed with stereotypy. Baseline rates of responding were determined at a standard current intensity of 30 uA, 100 Hz biphasic square wave for all animals (n=60). Following baseline testing one-half of the surgically prepared animals received intraperitoneal (i.p.) injections of d-amphetamine sulphate (7.5 mg/kg) twice daily for 5 consecutive days. The remaining half were chronically treated with saline. On test day (Day 6) the animals were subdivided further such that half the animals in each group were tested with a test dose of 0.3 mg/kg d-amphetamine sulphate while the other half received saline. This chronic drug treatment/test sequence was continued for 5 sessions (30 days). Rats chronically treated with saline and tested with d-amphetamine (0.3 mg/kg) showed comparable self-stimulation rates relative to animals chronically treated and tested with saline on all test days with the exception of test day 4. Among rats chronically treated with the test dose of d-amphetamine a significant facilitation of self-stimulation relative to the remaining groups was observed on four of the five test days. In contrast, rats chronically treated with amphetamine and tested with saline showed a depression of self-stimulation rates relative to controls. Thus within the same self-stimulation paradigm both response depression and sensitization were observed as a consequence of long-term amphetamine administration. It appears that when animals are tested with a low dose of amphetamine, which ordinarily has no behavioral consequences, ICSS responding is enhanced (sensitization), presumably due to a hyperactive dopamine system (receptor supersensitivity) resulting from chronic exposure to the drug. In the absence of the drug on test day the dopamine system remains hypoactive and consequently a depression of ICSS is observed.

PSYCHOPHARMACOLOGY


Phencyclidine (PCP), a leading drug of abuse, has anesthetic, analgesic, cardiovascular and psychotomimetic effects. In order to investigate the mechanisms of CNS actions of PCP, we studied binding of [3H]PCP to rat brain membrane preparations. [3H]PCP binds specifically and with high affinity (Kd ~ 1.5 x 10^-7 M) at a saturable class of binding sites. Specific binding constitutes approximately 70% of total binding at 0° and 33% of total binding at 37° (at 10^-7 M [3H]PCP). This binding can be displaced by nonradioactive PCP and a series of ten PCP analogs with relative potencies that closely parallel those determined in vivo in mouse rotorod (p < 0.005) and rat drug discrimination (p < 0.001) tests. Mucosal cholinergic Ligands inhibits [3H]PCP binding, but only at high concentrations (IC50 > 10^-4 - 10^-3 M) and in rank order at variance with that determined for their binding to muscarinic receptor sites or for their pharmacological potencies. Other centrally acting drugs including opiates are unable to displace specifically bound [3H]PCP at > 10^-4 M concentrations. In subcellular fractionation experiments, [3H]PCP binding is most enriched in the crude synaptosomal membrane fraction (3.3 times higher than in the mitochondria-synaptosome fraction; 5.1 times higher than in the whole brain homogenate). In regional binding distribution, [3H]PCP binding is highest in hypothalamus > caudate nucleus > frontal cortex > cerebellum > medulla/pons, amygdala. Binding of [3H]PCP to some peripheral tissues does occur but displacement of this binding by PCP analog fails to correlate with the CNS binding or with pharmacological properties. Trypsin, pronase, N-ethylmaleimide, iodoacacetamide, acidic pH, high temperature, and calcium reduce specific [3H]PCP binding. This suggests that its analogs may exert their CNS effects via binding to specific CNS receptor sites.

ABSTINENCE FROM L-ALPHA-ACETYLMETHADOL (LAAM), NOR-LAAM AND DINO-LAAM IN DEPENDENT RATS: EEG AND BEHAVIORAL CORRELATES. Gerald A. Young, George F. Steinfels and Nalid Xasan. Dept. of Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharmacy, Balt., MD 21201

We have previously studied and compared EEG and behavioral correlates during self-administration of LAAM, nor-LAAM and dino-LAAM in dependent rats (Young et al, J. Pharmacol. exp. Ther., in press). We have evaluated that the pharmacodynamic profile of LAAM differed from those of its two N-demethylated metabolites. LAAM disrupted behavior the least during self-administration. In the present report we will discuss these studies to a comparison of EEG and behavioral correlates during abstinence from LAAM, nor-LAAM and dino-LAAM in dependent rats. Adult female Sprague-Dawley rats were prepared with chronic intraventricular cannulae and cortical and temporalis muscle electrodes. They were made tolerant to and physically dependent on morphine by a series of automatic hourly injections that progressively increased in dose. Each rat was then trained to lever press in order to self-administer morphine (10 mg/kg/inj) on an FR-20 schedule of reinforcement. Upon stabilization of morphine self-administration, LAAM, nor-LAAM or dino-LAAM, each at a dose of 1 mg/kg, was substituted for morphine for an additional one to two weeks, and continuous EEG and EMG recordings were collected. Saline was then substituted for each of these narcotics which, thus, precipitated abstinence.

Upon abstinence from nor-LAAM, REM sleep was severely suppressed from the 18th through 28th hrs. In contrast, during abstinence from LAAM and dino-LAAM, REM sleep was moderately suppressed for two to three days. Furthermore, during abstinence from nor-LAAM occurred earlier and was more prolonged than for LAAM and dino-LAAM. The incidence of head shakes peaked earlier and was higher for nor-LAAM and dino-LAAM during abstinence than for LAAM. These results demonstrated further pharmacodynamic differences between LAAM and its two N-demethylated metabolites. Our findings suggest that in dependent rats abstinence from LAAM was least severe when compared with abstinence from nor-LAAM and dino-LAAM. (Supported by NIDA Grant DA 01500.)
PSYCHOPHYSICS
2287 ROLE OF POSITION SENSE IN DIRECTION DETECTION ON THE SKIN. D.A. Dreyer, G.H. Duncan*, and C.L. Wong*. Dental Research Center; Dept. of Oral Surgery, School of Dentistry; Dept. of Physiology, School of Medicine; Neurobiology Program, University of North Carolina, Chapel Hill, N.C. 27514.

Somatic position sense is thought to be of large capacity and to be optimal when the stimulating object is moving parallel to the skin's surface. An experimental model of this mode of stimulation was constructed that correctly identifies the direction of the brush stroke across the skin. Previous studies with this model have shown that the accuracy of direction discrimination improves with increasing length of skin contacted by the brush. This may be due to the increased surface area of stimulus contact or to the increasing separation of the extreme points of stimulus contact. The present study investigates these two possibilities.

Discrimination of brush stroke direction was assessed in normal human subjects for stimuli applied to the ventral forearm. The independent variables studied were the velocity of brush movement and the area of skin contacted by the brush. A servo-motor moved the brush at any of nine velocities ranging from 0.5 to 250 cm/sec.; velocity and direction of movement were randomized within each block of 180 trials. Area of skin contacted by the brush was controlled by plates with 0.5 cm wide apertures of different lengths (1-6 cm long) or two short slots split by a solid aperture. Thus the 4 cm. split aperture, for example, was identical to the 4 cm. slot (1-6 cm long) or two short slots split by a solid aperture. The subjects' reports of perceived brush direction were elicited by a forced-choice procedure.

The results of the present study indicate that, at slow brush velocities the ability to identify the stimulus direction was comparable for the continuous and split apertures. The amount of skin exposed to the stimulus in the split apertures was from 50-91% less than that exposed in a continuous aperture of comparable length. Thus the 4 cm. split aperture (4 cm. long) was comparable for the continuous and split apertures, although the amount of skin exposed to the stimulus in the split apertures was from 50-91% less than that exposed in a continuous aperture of comparable length. Thus at velocities that increase discrimination ability fell off sharply with both aperture types, but the performance decline with the split aperture was much more pronounced. These results are consistent with a stimulus model that emphasizes position detection in correctly identifying the direction of a moving stimulus. Although the total area of stimulus contact remained constant for all split-aperture plates, discrimination increased with increasing distance between the two apertures.

Supported by DE 02668, RR 05333, DE 07018, DE 00011 and the Alfred P. Sloan Foundation.

2288 NON-ANALGESIC EFFECTS OF NITROUS OXIDE ON SOMESTHETIC SENSITIVITY AND PERCEPTION OF SENSITIVITY. G.H. Duncan*, D.A. Dreyer, and J.M. Gregg* (SPON: J.S. Hanker). Dental Research Center; Oral Surgery, School of Dentistry; Dept. of Physiology, School of Medicine; Neurobiology Program, UNC, Chapel Hill, N.C. 27514

Subanesthetic levels of N₂O have been shown to produce impairments in auditory and visual acuity, psychomotor performance, short-term memory, and reaction time. A single study has documented changes occurring as a result of 25% N₂O impaired warmth and pressure thresholds in three subjects. It is generally acknowledged that the capacity to identify an object is optimal when the object and skin are in tangential motion relative to each other (identification of a coin by passing the fingers across its surface). This study investigates the effects of N₂O on somesthetic measures by an experimental model of tangential stimulus movement.

Ten volunteers were each tested under two conditions — 100% O₂ and 30% N₂O/O₂ 90%. All sessions were otherwise identical and consisted of 100 brush strokes presented to the thenar eminence. A servomotor moved the brush at any of 5 velocities ranging from 0.4 to 150 cm/sec.; velocity and direction of movement (either proximal to distal or the reverse) were randomized within each session. The area of skin contacted by the brush was 20 cm² with three rectangular apertures of 0.5 cm. width and 100 cm long. The subject reported the perceived direction of brush movement past the aperture by pushing a switch in the appropriate direction. The 30% N₂O group was significantly better than the 100% O₂ group at 20 cm².

The results of the present study indicate that, at slow brush velocities the ability to identify the stimulus direction was comparable for the continuous and split apertures. The amount of skin exposed to the stimulus in the split apertures was from 50-91% less than that exposed in a continuous aperture of comparable length. Thus at velocities that increase discrimination ability fell off sharply with both aperture types, but the performance decline with the split aperture was much more pronounced. These results are consistent with a stimulus model that emphasizes position detection in correctly identifying the direction of a moving stimulus. Although the total area of stimulus contact remained constant for all split-aperture plates, discrimination increased with increasing distance between the two apertures.

Supported by DE 02668, RR 05333, DE 07018, DE 00011 and the Alfred P. Sloan Foundation.
This study used Functional Measurement (FM) scaling procedures to demonstrate that subjects can scale and integrate pain that is produced by an electrical tooth pulp stimulus and symbolized by a word. Unlike conventional psychophysical scaling methods, FM includes a testable integration criterion that must be met before the scale is accepted. This method does not require physical measures of stimulus intensity and can produce direct scales of sensory input of non-metric stimuli such as words or pain experience. We required subjects to respond to the average of a metric and a non-metric stimulus set: 1) painful electrical stimuli (100 Hz, 1 sec trains of 100 Hz, 1 msec monophasic monopolar pulses) and 2) verbal descriptors (non-metric stimuli) of sensory intensity (weak, mild, moderate, strong, intense) and unpleasantness (annoying, uncomfortable, dreadful, horrible, agonizing) that symbolize different dimensions of the pain experience. Eighteen subjects received all possible pairs of 1) 5 tooth pulp stimuli ranging in equal log steps from pain threshold to tolerance, plus 2) 5 descriptors of sensory intensity or unpleasantness twice each for a total of 50 stimulus pairs. Each subject rated the average sensory intensity or unpleasantness of each tooth pulp-word pair by squeezing a hand dynamometer. The ability to average the perceptions and the validation of both the task and resultant scales can be tested by a statistical test (2 way ANOVA). A nonsignificant interaction term validates the task and resultant interval scales of both stimulus sets. Statistical analyses of sensory intensity responses showed significant main effects of tooth pulp stimuli (F (4,36) = 12.87 p<0.0001) and words (F (4,16) = 22.20 p<0.0001) and nonsignificant interaction (F (16,144) = 1.11 p<0.3483). Analyses of the unpleasantness responses also showed significant main effects of tooth pulp stimuli (F (4,28) = 4.84 p<0.0043) and words (F (4,16) = 12.99 p<0.0001) but the interaction was only marginally nonsignificant (F (16,112) = 1.73 p<0.0508). This result shows that subjects can use the intensity of a sensation or a word that symbolizes sensory intensity interchangeably in an averaging scaling task, supporting the use of verbal descriptors of sensory intensity in the assessment of pain. Unpleasantness scaling shows that these word scales are not integrated with actual stimuli as easily as sensory words. We conclude that subjects can integrate the pain produced by a tooth pulp stimulus with pain symbolized by a word and that this integration varies with the type of words used. FM methods may provide a new and promising tool for the assessment of pain experience.
REGENERATION
THE MECHANISM OF AXONAL REGENERATION IN CRAYFISH MOTOR NEURONS.
George D. Bittner, Michael S. Bouton*. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Previous studies have shown that the distal segment of a severed crayfish motor axon may survive for 100-250 days following separation from its cell body. Regeneration of the severed motor axon occurs by reconnection of the proximal stump with the surviving distal stump via mechanisms as yet unknown (Hoy, et al., Sci., 1967; Kennedy and Bittner, Cell Tiss. Res., 1974). Axonal regeneration in these animals has been associated with the presence of multiple profiles assumed to arise from the severed proximal stump and to grow within the glial sheaths of surviving distal segments (Nordlander and Singer, Z. Zellforsch, 1972). Similar phenomena have been reported in the leech (Van Essen and Jansen, Cold Spring Harbor Symposia, 1976; Frank, et al., J. Comp. Neurol. 1975; Carbonetto and Muller, Nature, 1977) and the earthworm (Birse and Bittner, Brain Res., 1976).

Several theories have been proposed to account for regeneration in these various preparations:


2) The proximal stump grows out as described above, but eventually reaches the target tissue and makes a new set of synapses. The original distal stump may or may not degenerate (Nordlander and Singer, Z. Zellforsch, 1972; Carbonetto and Muller, Nature, 1977).

We have initiated a series of experiments to determine more precisely the mechanism of regeneration in crayfish motor neurons. We are examining the distal stumps of severed motor axons at various times up to 400 days after regeneration. In reconnected cases, we have found more multiple profiles near the target muscle at 400 days than at 50 days after regeneration is first noted. We are injecting the proximal and distal stumps of regenerated motor axons to determine the origin and specificity of these multiple profiles.

WITHDRAWN BY AUTHOR
2297 MORPHOLOGY OF LAMINA IX NEURONS OF THE RAT THORACIC SPINAL CORD FROM DAY 14 OF GESTATION TO ADULTHOOD. John P. Cummings* and Demaris J. Stelzer. Department of Anatomy, SUNY-Upstate Medical Center, Syracuse, New York 13210.

Following spinal hemisection, Bernstein and Bernstein (Experimental Neurology 30:136-151) proposed that neurons proximal to the lesion "de-differentiate" to a more primitive state in which the neuron is once again capable of accepting synapses. From day 14 to day 20 following hemisection motor neurons were observed to have decreased dendritic fields, loss of dendritic branches and an increased number of dendritic varicosities along the entire length of their dendrites. The current study was undertaken to study the maturation of lamina IX neurons in the rat thoracic spinal cord from day 14 of gestation to adulthood so that the stages of differentiation of lamina IX neurons could be compared with the stages of "de-differentiation" described by Bernstein and Bernstein.

From day 6-14 to day 2-18 the primary dendrites of lamina IX neurons appear to be adult in number. However, they are short in length and have varicosities of uneven diameter. Hair-like structures protrude from these varicosities. From day 18-20 until postnatal day 2 the more proximal parts of the dendrites retain these varicosities, and, in addition are invested with long hair-like processes up to 5μm in length. These structures are also occasionally found on the bodies of the smaller dendrites. Beginning with postnatal day 12 there is a gradual shortening and reduction of dendritic spines on all parts of the neuron accompanied by a reduction in the number of tertiary dendrites. Beginning with postnatal day 24 and 30 lamina IX neurons resemble the "de-differentiated" cells reported by Bernstein and Bernstein in certain respects, but the stages of maturation are not directly comparable with the stages of "de-differentiation" until the adult morphology of lamina IX neurons is obtained between postnatal days 24 and 30.

Intra-axonal transport of 35S methionine labeled protein in lamina IX neurons resemble the "de-differentiated" cells described by Bernstein and Bernstein. From day E-14 to day E-18 the primary dendrites of lamina IX neurons appear to be adult in number. However, they are short in length and have varicosities of uneven diameter. Hair-like structures protrude from these varicosities. From day 18-20 until postnatal day 2 the more proximal parts of the dendrites retain these varicosities, and, in addition are invested with long hair-like processes up to 5μm in length. These structures are also occasionally found on the bodies of the smaller dendrites. Beginning with postnatal day 12 there is a gradual shortening and reduction of dendritic spines on all parts of the neuron accompanied by a reduction in the number of tertiary dendrites. Beginning with postnatal day 24 and 30 lamina IX neurons resemble the "de-differentiated" cells reported by Bernstein and Bernstein in certain respects, but the stages of maturation are not directly comparable with the stages of "de-differentiation" until the adult morphology of lamina IX neurons is obtained between postnatal days 24 and 30.

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2300 REGENERATION OF SPECIFIC NEUROMUSCULAR CONNECTIONS IN THE CRAYFISH. Pamela Els* and Samuel J. Veleg. Department of Biological Sciences, Dartmouth College, Hanover, N.H. 03755

In the projection of six motoneurons to a flat sheet of 40 muscle fibers, the superficial flexor muscle of the crayfish Procambarus clarkii, the probability of a given axon innervating a given muscle fiber is a simple function of the position of the fiber in the muscle sheet (Veleg & Wyman; J. of Neurophys.). A main feature of these connections makes this a very suitable system in which to study the regeneration of connections between identifiable cells. The third superficial root was cut as the nerve crossed between some muscle fibers, leaving the proximal stump anchored to the muscle and some neuromuscular junctions intact. The presence of these few remaining junctions facilitated nerve regeneration; in their absence, with an unattached nerve, degeneration of the proximal stump occurred. The animals were allowed to recover, and analysis was performed on isolated abdomens on a weekly basis by recording the spontaneous activity of the nerve with an extracellular oil electrode and measuring junction potentials (jp's) with 3M KCl microelectrodes. Regeneration was first found in animals 2-3 weeks post-op.

The small axons (1, 2 and 3) regenerated first, the large axons (4, 5 and 6) regenerate later. By 4 weeks post-op, the muscle was innervated by at least one of the axons (usually a small one) though there were 'holes' in the map, i.e., some muscle fibers were not innervated in regenerated animals. JP's of 10-20 mV in size, giant relative to controls (1-5 mV in size), were invariably found on a few muscle fibers. The region of these giant JP's varied with the post-operative age of the animals. Methylene blue staining revealed that the region of giant JP's corresponded to the area of the growing tip of the nerve. It was further noted that the regenerated nerve was initially a single process; branching was rare until several weeks post-op. The regenerating nerve frequently ran ventral to the plane of the muscle fibers, the superficial flexor muscle of the crayfish. Regeneration was complete, and comparable in connection specificity to control animals, by 9-10 weeks.

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2302 SYMPATHETIC RECEPTORS IN THE DEGENERATING AND REGENERATING VISUAL PATHWAY. Andrew Francis* and Norman Schecter. Department of Neurology, Cornell University Medical College, New York, 10021 and Department of Psychiatry and the Long Island Research Institute, UMD, Stony Brook, New York 11794.

After optic nerve crush, regeneration of connections in controls were not innervated in regenerated animals. JP's of 10-20 mV in size, giant relative to controls (1-5 mV in size), were invariably found on a few muscle fibers in controls. The region of these giant JP's varied with the post-operative age of the animals. Methylene blue staining revealed that the region of giant JP's corresponded to the area of the growing tip of the nerve. It was further noted that the regenerated nerve was initially a single process; branching was rare until several weeks post-op. The regenerating nerve frequently ran ventral to the plane of the muscle fibers, the superficial flexor muscle of the crayfish. Regeneration was complete, and comparable in connection specificity to control animals, by 9-10 weeks.

(Supported by NIH Grant NS 13800 to SJV)


Mithoracic spinal cord transaction in bullfrog tadpoles produces behavioral deficits that persist and in some cases are irreversible up to 90 days post-lesion. The laminae of the spinal cord adjacent to the lesion do not degenerate. Following transaction of the mid-thoracic spinal cord, gross anatomical continuity of the cord is restored in 1-2 weeks. For periods up to 60 days postoperative, however, righting reflexes are sluggish, responses are difficult to evoke by visual stimuli, and there is little swimming in the absence of stimulation. Injection of HRP into the lumbar enlargement of the previously operated tadpole reveals heavy labeling of fibers caudal to the thoracic transaction site but no fibers bridging this region are labeled. While in the unoperated tadpole, cell bodies in the vestibular nuclei and brainstem reticular formation are consistently found, cells in these areas are not labeled in the previously transacted tadpoles. Electrophysiological evidence indicates functional connection between the previously severed halves in tadpole stages may be mediated by short axons of propriospinal neurons crossing the gap.

Transected tadpoles held through metamorphosis showed the same pattern of behavioral preservation of metabolism. Following the 2-3 week metamorphic period, these animals showed behavioral recovery such that they were indistinguishable from unoperated controls. The righting reflexes were intact and normal. The light reflexes were brisk, and scratch reflexes were properly directed and of normal threshold. HRP injection into the lumbar enlargement labeled the targets in the brain that bordered the transaction site. Somas were labeled in the vestibular nuclei and throughout the reticular formation as far rostral as the midbrain.

Two possible explanations of the differences between tadpoles and juvenile frogs can be considered. First, the stimulus necessary for regeneration of long to medium tract is present only during early metamorphosis. Alternatively, different brainstem neurons might project to lumbar regions in tadpole and frog. During metamorphosis, these new descending connections could develop in both unoperated and previously transacted tadpoles to form the substrate of normal behavior in both groups of animals.

(Supported by NSF Grant BNS 79-10528 and USPHS Grant NS 14899)


The ability of 8,9-dihydroxy adenosine 3',5'-monophosphate ([buty]cAMP) to stimulate nerve development in vitro prompted us to study the effect of the nucleotide on in vivo degeneration and regeneration of crushed rat sciatic nerves. The animals received daily intramuscular injections of either (buty)cAMP (50mg/kg/day) or 0.9% saline after operation and were tested daily for the return of somatosensorimotor function (SMF). An index of SMF was obtained by placing the foot of the lesioned limb over the escape hole in the high intensity light box. The time required for foot withdrawal was recorded to the nearest second. (buty)cAMP and saline-treated rats showed little difference in degree of SMF from 10 days, but by day 12, the response times for the nucleotide-treated animals appeared to be completely recovered by day 16, while saline treated rats did not exhibit full recovery until day 26. To investigate the morphological changes that accompanied this functional recovery, rats were sacrificed 3, 10, 18, and 32 days following crush and their nerves prepared for transmission and scanning electron microscopy. These observations, fiber counts and measurements of fiber size indicated that (buty)cAMP accelerated the initial processes of Wallerian degeneration and enhanced the rates of regeneration and maturation of the peripheral nerve fibers. In general, the crushed nerves of nucleotide-treated animals appeared 7-8 days more advanced in their degenerative and regenerative processes than the saline controls. Previous workers who found no effect of (buty)cAMP on nerve regeneration probably did not allow enough time to pass for these effects to express themselves, since morphological and physiological differences between regenerating nerve fibers of control and saline treated rats appeared only after 8-10 days post lesion.

(Supported by NIH NS-11299)

2305 MORPHINE-ENHANCED REGENERATION OF CENTRAL NORADRENERGIC NERVES FOLLOWING 6-OHDA DAMAGE. Craig T. Harston, Judy C. Hardin* and Risteed M. Kostrzewa. Dept. of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, Tennessee 37601.

The locus coeruleus, which provides the noradrenergic innervation to the cerebellum, is known to be innervated by serotonergic, substance P- and endorphin-containing neurons. Because of the possibility that these neurosecretory substances from these nerve terminals could serve as neuromodulators on the development of the locus coeruleus, we investigated the effect of morphine, an endorphin-mimetic, on development of the noradrenergic terminals in the cerebellum. Morphine sulfate (20 μg/g i.p.) was injected into intact and neurotoxin-treated (i.e., 6-hydroxydopamine [6-OHDA]) 60 μg/g i.p.) rats on the day of birth or 3 days after birth. Morphine treatment potentiated the recovery of NE levels in the cerebellum and pons-medulla of the rats treated with 6-OHDA on the third day after birth. No effect of morphine was found on the NE content of the hippocampus of control or 6-OHDA-treated rats. The cerebellar effect was morphine dose-related (0.25 - 20 μg/g i.p.) in rats treated on the day of birth and was apparent by 14 days of age. The morphine-induced increase of NE in the cerebellum was corroborated by increased density of histofluorescent fibers (glyoxylic acid method) in the cerebellum of adult rats. These data show that acute morphine treatment potentiated the recovery of the noradrenergic system in the brain following neonatal damage with 6-OHDA. These results suggest that neurosecretory agents (e.g. endorphins) could modulate regeneration of damaged neural systems. (Supported by the National Foundation March of Dimes)

2306 REGENERATION


In passag, whole nerve recording of activity in As fibers in one branch of the femoral cutaneous nerve of a cat was made with a monopolar hook electrode 16 months after the branch had been ligated with 6-0 silk thread. At the time of the recording, there was no trace of the ligature and no sign of damage to the nerve when examined under 25× magnification. The nerve branch innervated 18 type 1 receptors (domes), each of which produced a typical continuous, but irregular train of discharges when stimulated with its long axis oriented dorsoventrally. The walls are composed of cells in pseudostratified arrangement usually with two, sometimes three, layers of nuclei. After injury the canal changes shape and orientation so that a flattened wall, as originally seen laterally, "faces" the lesion. The arrangement of the cells is no longer uniform. The lateral wall often appears to have only one cell layer, whereas the soles have an aggregate or "stratification" of many cells. The walls lose their uniformity in thickness and cellular arrangement. Preliminary autoradiographic findings show that at 2 days post-operative 5.75% of the cells are labeled and by 5 days post-operative, 0.85% of the cells are labeled and by 7 days or more the proliferative activity is at the control level. (Supported by USPHS Grant NS 04761.)
FOREIGN NERVE INNERVATION OF A MUSCLE FIBER POPULATION IN THE CRAYFISH. William P. Hunt* and Samuel J. Velez. (SPON: H. L. Borison) Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The six motoneurons that innervate the 40 muscle fibers of the superficial flexor muscle of the crayfish Procambarus clarkii can regenerate their connections within 9-10 weeks with a high degree of specificity (Bly & Velez, Neurosci. Abstr. 1979). Experimental manipulation of this system during regeneration can give us insight on the processes that influence the formation of specific connections between identifiable cells. The third superficial root that innervates the left superficial flexor muscles of the third segment (to be referred to as 'the foreign nerve') was transplanted to the contralateral superficial flexor muscles of the same segment. In some experiments, the denervation of the recipient muscle was left intact, while in others the muscle was denervated. Animals were left to recuperate, and analysis was performed on a weekly basis by monitoring the spontaneous activity of the innervated muscle. In both cases, the small axons appear to grow first, followed by the large axons. Initial innervation spreads over the entire field leaving some 'holes' in the map, just as was observed when the original nerve innervates the muscle after it has been cut (Bly & Velez, ibid). The initial synapses made by the foreign nerve are characterized by gigantic j.p.'s (10-20 mV in size when compared to 1-5 mV in size in control animals), little facilitation (when comparing j.p. sizes at 1 Hz and 10 Hz stimulation) and some signs in firing (no j.p.'s produced even though the axon is firing). The presence of the original nerve in a muscle fiber seems to have no effect on the ability of the foreign nerve to make connections with that fiber as j.p.'s produced by the foreign nerve were the same as the map of the original nerve on the same fiber. Innervation by the foreign nerve is complete across the muscle field for the first growing axons by 8 weeks, and the activity map appears to be the same as the map of the original nerve. 

(Supported by NIH Grant NS 13600 to SJV)


Long term cultures of superior cervical ganglion (SCG) neurons can now be obtained from not only embryonic but also postnatal rats, including fully adult animals (Nature 267: 536, 1977). This allowed the study of the relative growth capacities of neurons from rats of different ages. Explants of the SCG from 21-day embryo and postnatal rats were grown in the same dish on an air-dried collagen substrate and fed either standard feed containing human placental serum and embryo extract or feed added antigenic agents to eliminate nonneuronal cells. All feeds contained NCF. The onset and rate of neuritic outgrowth were monitored sequentially by photography and the data analyzed by computer. Explants from perinatal rats exhibited vigorous neuritic outgrowth (by 6 hours) with an average growth rate of 35m/d. The neuritic outgrowth from explants of adults was delayed in onset (56 hours) and lengthened at 19m/d. The growth pattern of neurons from 5 week old rats was intermediate between the perinatal and adult (32 hours; 21m/d). When antitotic agents were present in the feed the rate of growth was slowed in all age groups. Further studies are in progress to determine whether antitotic agents act directly on the neurons or by removing immunological elements of the system will also allow the evaluation of other possible influences on regenerating neurites including that of NCF.


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Non-specific axonal growth in the optic tectum of adult frogs. Elliot J. Kaplan* and Carmine D. Clements. Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, California 90024.

The reestablishment of appropriate synaptic connections in regenerating CNS tissue is a requisite to functional recovery. That some form of recognition exists between growing axons and their target cells has been postulated in numerous studies of the retinotectal projection of several species. However, the accuracy of this recognition is not yet understood. Recent experiments have shown that during regeneration of the optic nerves of goldfish, spermidine (Spd) is axonally transported (AT) and co-localized with axonal RNA. The presence of the original nerve in a muscle fiber seems to have no effect on the ability of the foreign nerve to make connections with that fiber as j.p.'s produced by the foreign nerve were the same as the map of the original nerve on the same fiber. Innervation by the foreign nerve is complete across the muscle field for the first growing axons by 8 weeks, and the activity map appears to be the same as the map of the original nerve.

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(Supported by NIH Grant #02987 from NIH-NEI.)
ANOMALOUS SYMPATHETIC TERMINALS ON HIPPOCAMPAL VASCULARIZATION FOLLOWING FIMBRIAL DAMAGE. Rebekah Loy and Robert Y. Moore. Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093

Following lesions of the septum or fimbria, anomalous, noradrenaline (NA)-containing axons grow into the hippocampal formation (HF). These fibers appear to originate from a normal sympathetic innervation of the longitudinal hippocampal arteries. Fluorescence histochemistry reveals that the large calibre NA-containing axons usually extend to the apical portion of the arachnoid space of the lateral ventricle. Two weeks following a fimbrial lesion, fluorescent axons also accompany penetrating branches of the lateral choroidal arteries and terminate within the arachnoid space of the lateral ventricle. These axons may also extend to the hippocampal fissure and the fimbria-dentate fissure.

In order to determine the localization of any synaptic contacts formed by this anomalous sympathetic innervation, we have compared brains from normal rats and rats which 15-90 days previously had received perfusions of the fixative. Some animals of each group received an intraventricular injection of 5-hydroxodopamine (5-OHDA) 1 hr prior to perfusion, to produce small granular vesicles (5-OHDA), which were shown to contain both noradrenaline and dopamine. In another experiment, the lateral ventricle was transected to its longitudinal axis, leaving intact the meninges, choroid plexus, and vascularization within the lateral ventricle.

Electron microscopic examination of normal rat brains showed many varicosities containing 5-OHDA's adjacent to the basal lamina surrounding the smooth muscle of ventricular arteries. In lesione Brains similar 5-OHDA-containing varicosities appear immediately adjacent to the basal laminae of smooth muscle surrounding subpial arterioles and arterioles. Other 5-OHDA-containing varicosities appear adjacent to the basal laminae of arterioles for or of endothelial cells directly. Such varicosities are present within the neuropil as well, however these are most common in the subvascular neuropil. Thus, these are most common in the subvascular neuropil. Such anomalous innervation may have significant consequences for the regulation of cerebrospinal fluid flow and capillary permeability following CNS damage.

Supported by NINCDS Grant NS-14372. We would like to thank Ms. Lauralee Butler for excellent technical assistance.
SYMPATHETIC REGENERATION INTO THE HIPPOCAMPUS: EFFECT OF LESION SITE. Teresa A. Miles* and Rebekah Loy. (SPON: S. Barondes).

Peripheral nervous system (PNS) appear to innervate the hippocampus and have found that existing, undamaged afferent projections are essential prerequisites for recovery of function in many neuronal systems. Previous experiments have examined reorganization of the hippocampus following selective deafferentation, and we have found that undamaged, immature systems invade the partially deafferented area following entorhinal cortex lesions. Recent studies have shown in addition to this intrinsic reorganization, that sympathetic fibers of the peripheral nervous system (PNS) appear to innervate the hippocampal dentate hilar area and stratum lucidum of C3 following destruction of the fornix or anterior hippocampus. The pattern exhibited by these PNS fibers is selective and does not correspond to any other innervation patterns from hippocampal afferent projections. The present study investigates more fully the conditions necessary and sufficient to produce this anomalous growth of PNS fibers into the hippocampus following injury.

Various combinations of afferent and efferent pathways of the rat hippocampus were selectively destroyed. After 10 to 30 days, the brains of these animals were analyzed using the glyoxylic acid method of fluorescence histochemistry. We would like to thank Lauralee Butler for technical assistance.

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The principal pathology common to all forms of Parkinson's disease is neuronal cell loss and gliosis in the zona compacta of the substantia nigra (SN). As a result of the loss of these cells, there is a decrease in the concentration of dopamine in the striatum. Replacement of missing DA is the conceptual backbone of current therapy. The peripheral administration of L-DOPA and dopaminomimetic agents unfortunately is only partially effective and is associated with side effects. Since the ideal therapy would be a circumscribed infusion of DA into DA-deficient areas of the brain, we wondered if it would be possible to achieve this result by grafting DA-containing cells into these areas.

To test our idea, we used the rotting rat model of Parkinson's disease. In this model the SN is lesioned unilaterally by 6-hydroxydopamine. Ipsilateral to the lesion there is a reduction in the concentration of DA in the striatum and an electrophysiological, biochemical and behavioral supersensitivity. The latter is manifest by contralateral rotational response to the DA agonist, apomorphine. We hypothesized that if we were able to graft DA-containing and secretory cells from the SN to the DA-denervated striatum, there would be a reversal of these well described events.

Following the grafting of fetal rat SN (17-day gestation) to SN-lesioned adults rats, we found: (1) Histofluorescently, all SN grafts survived and contained catecholamine (CA) and indoleamine-producing cells. The CA containing cells had migrated and sent axons into the brain tissue adjacent to the graft with heaviest innervation into the caudate nucleus. (2) Biochemically, all the grafts contained DA; the amount of DA in the innervated caudate was increased in the animals with grafts, especially in areas adjacent to the graft. (3) Behaviorally, the apomorphine induced turning was reduced in SN grafted animals. This is consistent with the improved performance of the limbs of SN-lesioned goldfish in the host caudate nucleus, the behavioral and microscopic picture observed at 5-9 months post-grafting was unchanged from that observed at 2 months post-grafting. There was no evidence of tissue rejection.

Current efforts are being undertaken to determine if similar grafting techniques can be used in the non-human primate.


The optic axons of goldfish regenerate when crushed intra-orbitally. They reach their target, the tectum, within 5-7 weeks post-crush. Previous studies have suggested that these regenerating axons branch repeatedly so that an excessive number of synaptic connections are formed. The goal of this study was to determine if these regenerating axons can innervate areas of the tectum and should then be potentially available to reinnervate tectal neurons.

In this study the right optic nerve of 6-8 inch goldfish was crushed within the orbit. Animals were allowed to survive from 6 days to 8 months, perfused, and the optic nerves, tracts and tecta prepared for EM. Observations on the organization of the regenerating axons were considered in terms of: (1) the percent of the axons which are myelinated, (2) the amount of glial cytoplasm present in the nerve distal to the crush site, (3) the numbers and types of synapses on the axons, (4) the size of regenerating axons, (5) the percent of them which are myelinated, 3) the amount of glial cytoplasm present in the nerve distal to the crush site, (6) the size of regenerating axons, (7) the number of types and neural and non-neural elements present in the tectal neuropil (SGS) during regeneration suggest the following: The normal optic nerve is composed almost entirely of myelinated axons which are organized in fascicles. At 2 weeks post-operatively large numbers of gial cells are present between the fascicles of degenerating fibers. Within the fascicles the degenerating fibers and glial cells are located peripherally, while bundles of regenerating axons form the core of the fascicle. Estimates at this time show that the number of regenerating axons profiles increases over the normal number of profiles within 5-7 weeks. During the ensuing month the number of axonal and glial debris and glial cytoplasm decreases markedly and the size of regenerating axons increases; the number of regenerating axons appears to remain relatively stable. 6 months post-operatively the optic nerve has begun to regain its preoperative appearance.

Regenerating axons entered the tectum by 5-7 weeks post-operatively. The number of the unmyleinated axons in SGS and SFGS is greatly increased but the number of axon terminals making synapses is much greater than normal. By 6 months post-operatively the number of axons in the SFGS is still greater than normal but the number of axon terminals approaches normal level. In animals in which all axons have degenerated, axonal sprouts are formed and grow into the tectum, they do not hyperinnervate the SFGS. This suggests that the tectal cells have the capacity to accept only a limited number of retinal connections.

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In some regions of the vertebrate nervous system, such as mammalian autonomic ganglia, damaged axons can regenerate and restore appropriate functional connections. On the other hand, regeneration elsewhere, for example motor innervation, is generally non-specific and inappropriate for the tissues receiving the foreign axons. We are investigating reinnervation of the parasympathetic cardiac ganglion of the frog to elucidate the cellular mechanisms underlying the restoration of appropriate innervation after nervous tissue damage. We have shown (Neuroscience Abstracts 4, 533, 1978) that hypoglossal motor axons can synapse with an innervated cardiac ganglion. In these experiments we report that when vagal preganglionic axons reinnervate the cardiac ganglion after hypoglossal innervation has been established, they take over at a considerable stage of regeneration.

The surgical operation for these experiments consisted of sectioning both vagi and suturing the left hypoglossal to the distal stump of the left vagus. Regenerating hypoglossal axons innervate parasympathetic neurons in the cardiac ganglion in about 8 weeks. After a further delay of a few weeks, we allowed axons from the right vagus nerve to reinnervate the ganglion. At intervals from 8-52 weeks after the operation, animals were killed and cardiac ganglia removed. Neurons were impaled with microelectrodes to record synaptic input from hypoglossal and/or regenerating vagal axons. Ganglia were subsequently fixed for light and electron microscopy.

Animals were divided into three groups correspond to early (8-20 weeks), middle (21-30 weeks) and late (31-52) stages of reinnervation. The proportion of neurons receiving vagal innervation at these times were 73±5%, 57±23% and 14±10% (N=15 animals, values are means ± s.e.m.). These data show (1) regenerating vagal axons restore functional connections despite the presence of foreign innervation, and (2) hypoglossal innervation disappears completely in the hypoglossal regeneration defects.

Electron microscopy show that vagal terminals reestablish synapses on the axon and cell body region of ganglion cells. These synapses were comparable to those in ganglia from unoperated animals. Hypoglossal terminals, on the other hand, contact only the axons of parasympathetic neurons. We speculate that the difference in reinnervation of foreign and native systems is fundamental to the observed selective in the reinnervation of cardiac ganglia, and that the glial cell which envelopes the ganglion cell body acts as a barrier to inappropriate terminals but not to native ones.
REGENERATION

2220 ANALYSIS OF THE TECTAL PROTEINS IN THE VISUAL PATHWAY OF GOLD-FISH BY 2D GEL ELECTROPHORESIS. Wolfgang Gutachter1*, Andrew Prasciutti1, and Hans Rehbein. (1) I. Paul. Departments of Biochemistry and Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, New York 11794; and (A.P.) Cornell University Medical College.

In goldfish, after optic nerve crush, regeneration and specific reconnection of the optic nerve to the tectum occurs. This paradigm is emerging as an important model system for the study of neuronal specificity. One critical aspect of this problem is the role of unique or specific proteins involved in the reconnection process. Eight-sided enucleation or Intracortical optic nerve crush was performed on large (10-12 cm) common goldfish maintained on a standard diurnal cycle. Protein separation patterns were obtained from the affected (contralateral) as well as the intact (ipsilateral) tectum by two-dimensional isoelectric focusing-gel electrophoresis described by O’Farrell. After coomassie blue staining, analysis of the gel patterns revealed at least one protein, having a molecular weight of 57,000, whose presence paralleled optic innervation. This protein was lost from the tectum after enucleation, and appeared to be present in tecta after optic nerve regeneration and reconnection.

Further analysis indicated that the protein could not be detected in whole brain preparations or spinal cord, forebrain, cerebellum, vaginal lobes, or medulla. The protein was detectable only in the optic nerve and tectum of the goldfish. The protein was associated with a membrane fraction of the lateral nerve and with both a membrane and soluble fraction of the tectum. With known standards it did not comigrate with either actin or tubulin.

Studies are in progress to determine the site of synthesis and possible axonal transport phenomena of this protein.

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2221 REINNERVATION OF SCIATIC NERVE GRAFTS TO THE RAT SPINAL CORD. Peter M. Richardson, Ursula N. McGuinness* and Albert J. Aguayo. Department of Anatomy and Neurosurgery, McGill University, Montreal, Canada.

In young adult female rats, a segment of the rat spinal cord was resected and replaced by an autologous sciatic nerve graft. Twenty-four animals were sacrificed from 10 days to 4 months later and the grafts and their junctions with the spinal cord were examined with the light and electron microscopes. All grafts of more than 3 weeks duration were richly innervated with myelinated and unmyelinated axons even in 8 animals in which the spinal roots entering thegraft site were avulsed together with their ganglia. At the border of the CNS tissue, dome-shaped structures were present, each filled with astrocytic processes and surrounded by a lamina. Myelinated and unmyelinated fibres frequently lay within these cellular domes. Occasional nodules of schwann were seen in which one or more had peripheral myelin and the other had central myelin.

In a further group of rats, horseradish peroxidase was injected into the spinal cord immediately adjacent to the graft (of 2-4 months duration). Two days later some neurones in the spinal cord on the other side of the graft were labelled with the enzyme. These results suggest that at least some axons of the central nervous system can grow into a peripheral nerve graft and be ensheathed by Schwann cells.


Central monolaminergic fibre systems have the ability to sprout and regenerate in response to mechanically or chemically induced injury (e.g. Katzman, Bjorklund, Owman, Stenevi and West, Br. Res. 25:579, 1971). One model which has proven particularly suitable for demonstrating regeneration is that in which peripheral tissue, particularly the iris, is implanted into the brain or spinal cord (for a review see Svendgaard, Bjorklund and Stenevi, Adv. anat. embryol. Cell Biol. 51:7, 1975). In previous work, identification of some regenerating fibers as adrenergic has been established by histochemical, histochemical and ultrastructural techniques. The purpose of the present study is to demonstrate by histochemistry and by autoradiography that axons projecting from the locus coeruleus are capable of regeneration into iris tissue implants placed into the caudal mesencephalon.

Briefly, heterologous iris implants were inserted in the caudal mesencephalon in the region of the dorsal tegmental bundle in female rats previously subjected to ipsilateral superior cervical sympathectomy. Previous histoautoradiographic work had shown that the implantation procedure severed the dorsal noradrenergic bundle (Katzman, Broida and Raine, Br. Res. 138:423, 1977). In order to observe regeneration of adrenergic fibers into in situ implants, half of the specimens were freeze dried and treated according to the histochemical fluorescence method of Falck and Hillarp. To establish the origin of these regenerating noradrenergic fibers, the remainder of the subjects received injections of 3H-lucine into the ipsilateral locus coeruleus 2 days before sacrifice and the brains were prepared for autoradiographic analysis according to the method of Cowan, Gottlieb, Hendrickson Price and Adelsley, Br. Res. 32:1271, 1972. We observed the presence of many bright green fibers running from the dorsal catecholaminergic tract and penetrating into the iris tissue implant. Microspectrofluorometric analysis indicated that these fibers were noradrenergic. These findings, which confirm the initial observations of Bjorklund and Stenevi (Br. Res. 31:1, 1971), indicate that noradrenergic axons are capable of sprouting and regeneration after injury. The results of the autoradiographic study, which will be presented in detail, suggest that some of these fibers originate in the locus coeruleus.
The time course of increased glucose utilization in hypoglossal nucleus neurons during regeneration. Philip A. Singer and Sharon Nahler. Dept. Neurology, Kansas City Veterans Medical Center, Kansas City, Mo. 64128.

2234. NUCLEUS NEURONS DURING REGENERATION. Philip A. Singer and Sharon Kansas City, Mo. 64128.

The time course of increased glucose utilization in hypoglossal nucleus neurons during regeneration. Watson J. Physiol. 180:741, 1965). Both groups have shown increased uptake of 14C2 deoxyglucose by hypoglossal motor neurons beginning 48 hours following axotomy. The energy metabolism during this reaction is controversial however, with some single neuron studies showing increases in oxidative metabolism in both axotomized and control nuclei (Kammerger and Sjostenrand. Acta Physiol. Scand. 67:16, 1966) and others showing decreases. Watson J. Neurochem., 13: 849, 1966). Numerous histochemical determinations of respiratory enzymes likewise show both increases and decreases. However, glycogen disappearance from large anterior horns cells 72 hours after axotomy (Casas and Engel. Science 171:198, 1971) indicates increased glucose use. Other studies suggest that glycolysis (Watson J. Physiol. 198:77, 1968) and the hexose monophosphate shunt (Mandy Arch Neurol. 18:62, 1968) are increased.

We used the 14C2 deoxyglucose technique to study glucose utilization. The left hypoglossal nerve was sectioned at the level of the carotid artery on one side. Following axotomy, the animals were sacrificed 45 minutes later and frozen sections of the brainstem exposed to x-ray film. The autoradiographs were enlarged and the optical densities of the sectioned areas of the right and left nuclei determined. There was a marked increase in glucose utilization 48 hours after axon section which was maximal at 72 hours but unaltered at 14 days. This data indicates that when the cells utilize very little glucose and energy metabolizing enzymes may be the initial target of the signal for regeneration. The studies listed above suggest that glycolysis and the hexose monophosphate shunt may be the only pathways for this increased metabolism. However, the magnitude of the increase suggests that oxidative systems may also be increased.


It is well established that postnatal undernutrition reduces the total number of cells and the growth of Purkinje cell dendrites in the cerebellum. The purpose of this study was to investigate the capacity for regrowth of Purkinje cell dendrites previously shrunk by undernutrition.

This study utilized a total of 22 B6D2F1 hybrid mice which were randomly cross fostered to enable littermate pairs, matched for sex and prenatal and postnatal age between 0 to 40 days of age with different nutritional intake. Control litters were normally nourished while experimental litters were undernourished before weaning by the method of enlarging nursing litter size and after weaning by restricting access to mouse chow. At 60 days of age, undernourished animals weighed 70% less than controls. At this point rehabilitation was initiated by reinstating ad lib access to mouse chow. One group of experimental and control mice were sacrificed at 45 days of age and another at 250 days of age for a morphometric cerebellar analysis utilizing the Golgi-Koppe technique.

At the end of 145 days of age, partial recovery of all parameters was noted with body weight (-1.1%), brain weight (-1.2%) versus sectional area (-1.5%), granular layer area (-1.9%), molecular layer area (-1.1%), Purkinje cell field areas (-4.9%) and Purkinje cell total length (-9.6%). By 230 days of age a significant difference was still noted in brain weight (-9.0%), versus area (-8.5%) and granular layer (-11.8%) however, almost complete recovery of all parameters was noted of the molecular layer (-5.8%), Purkinje cell dendritic field area (-1.4%) and total dendritic length/Purkinje cell (-1.3%). It is remarkable that the cerebellar molecular layer and Purkinje cell dendrites of rats maintained by ad lib nutrition can be induced to regenerate in the adult. Since it is unlikely that new granule cells could be formed during the rehabilitation period it would appear that parallel fibers must be capable of lengthening and producing new presynaptic processes which induce further growth in the Purkinje cell dendritic tree. These data offer evidence that the Purkinje cell dendritic tree retains a capacity for plasticity into the adult period.

(Supported by NIH Grant #NS 10657).

2236. INFLUENCE OF CULTURED NEURAL AND NON-NEURAL CELLS AND COLLAGEN ON RAT SCIATIC NERVE REGENERATION. Barry H. Smith, Vivian A. Betton* Calvin S. Hawkins* and Arthur M. Banks* SNB, NINCUS, NIH Bethesda, Maryland 20024.

Several factors influence the success or failure of peripheral nerve regeneration. An understanding of the cellular factors involved have been utilized a veined graft to repair rat sciatic nerves. The vein graft serves as a chamber into which to place various tissues such as nerve, cultured human skin, and adult, neural or non-neural cells to determine which effects they may have on nerve regeneration.

We have utilized the Osmond-Mandel rat and inbred Fisher rats have been utilized. The sciatic injury procedure is performed by transection in the thigh and the vein graft is taken from a segment of the inferior vena cava into the femoral vein prior to suturing the graft to the vein. The graft is maintained in place by microcrystalline collagen ('"Avitene") is placed in the vein graft and disappear after several weeks. We are currently investigating the influence of the various rat fetal and adult lines on the regeneration process.


The superior cervical sympathetic ganglion (SCG) of rats was studied during the process of synaptic restoration which follows neonatal preganglionic chain section. Litters of rats were examined at four and six months, and the left SCG was sectioned, a procedure which we have previously shown results in the eventual recovery of normal synapse numbers in the SCG. The third group served as unoperated controls. The litters of rats were killed on days 1, 7, 15, 21 and 29, and the left SCGs were processed for electron microscopy. Quantitative ultrastructural methods were used to yield estimates of the number of synapses and postsynaptic membrane specializations (PMS) unapposed by a presynaptic terminal (presumably vacated PMS).

In both groups of operated animals, 90% of the synapses were lost by the 4th postoperative day. The remaining synapses presumably belong to intrinsic ganglionic neurons. In the rats in which the PCC was sectioned, restoration of synapse number began at 21 days. However, in the animals in which the PCC was removed, there was no restoration of synapse number. Thus, the intrinsic ganglonic neurons failed to sprout to produce additional synapses in response to the massive deafferentation caused by removal of the preganglionic input.

The number of vacated PMS equalled only about 10% of the number of lost synapses in both groups of operated neonatal animals on the 4th postoperative day. This is in contrast to the findings in the deafferented SCG of the adult rat, where vacated PMS equal about 50% of the number of lost synapses.

These findings demonstrate that the mechanism of synaptic restoration in the neonate differs from that in the adult. In the adult, the regenerated presynaptic terminals reestablish contact with the vacated postsynaptic sites. In the neonate, however, the vacated postsynaptic sites and synaptic restoration appears to recapitulate normal synaptic development.

Supported by NS 10657 and NIH Grant #NS 13768.


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2239. INFLUENCE OF CULTURED NEURAL AND NON-NEURAL CELLS AND COLLAGEN ON RAT SCIATIC NERVE REGENERATION. Barry H. Smith, Vivian A. Betton* Calvin S. Hawkins* and Arthur M. Banks* SNB, NINCUS, NIH Bethesda, Maryland 20024.

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that the mature astrocyte may constitute the principal impeding properties. The results of this investigation suggest regenerating nerve fibers even though the initial injury was situated some distance away from the root entry zone and did not characterize of a neuroma. The fact that astrocytes block the organelles characteristic of regenerating axons, some have terminated axons at the root entry zone end blindly. Many contain Colchicine has been shown to produce temporary chromatolysis, nucleolar enlargement, retraction of presynaptic terminals and loss of synaptic transmission when applied locally to neural tissue. These effects are reversible in 3 to 4 weeks. We wanted to investigate the possibility that during recovery plasticity might be present. To this end, a solution of colchicine (2µl mole) was applied to the visual cortex of one hemisphere in adult cats for 30 minutes. After recovery from surgery the cats were exposed to goggles, with vertical lines for one eye and horizontal lines for the other, 4 hours every day for a period of 2 months. The animals were kept in a dark room at all other times. At the end of two months, single cells were recorded from the visual cortex of the colchicine-treated hemisphere and from the untreated one, which served as control, and their receptive fields were mapped. The data shows that extensive reorganization of receptive field orientation, sensitivity and binocularity took place in the colchicine-treated hemisphere. Here, cells were mostly monocular and responded to bars of the same orientation viewed by the driving eye during recovery. A few cells were found that responded to vertical lines when tested through the eye that had viewed horizontal lines, and also to horizontal lines when tested through the eye that had viewed horizontal lines. Thee cells were never found normally. Cells in visual cortex of the untreated hemisphere exhibited normal properties and receptive field types, i.e., they were mostly binocularly driven, and were distributed evenly for terms of orientation sensitivity. Histological analysis indicates that some neuronal rearrangement had taken place in the treated cortex. The results will be discussed in terms of the interaction between experience and the induced neuronal modifications.

**2329**


Following ablation of a hemitegment in goldfish, the severed optic nerve fibers regenerate and innervate the remaining ipsilateral hemitegment. Both the pattern of innervation and the pathways taken by regenerating fibers to reach the IOT were examined with autoradiography. Two types of surgical preparations were used: Type I - both eyes intact and one hemitegment ablated on the same side of the fish and Type II - both eyes intact and one hemitegment ablated.

Labeling of retinal ganglion cells in Type I fish revealed a normal laminar pattern of innervation in most, but not all fish. The majority of the regenerating nerve fibers appeared to reach the IOT via the ipsilateral optic tract (IOTr). In contrast, the IOT projection in Type II fish was discontinuous and few fibers appeared to reach the IOT via the IOTr. Discontinuity of the IOT projection in Type II fish may have resulted from the presence of the contralateral optic nerve fibers mechanically blocking the regenerating nerve fibers from reaching the IOT via the IOTr. Thus, the discontinuous IOT projection may in part be a consequence of a large number of fibers failing to reach the IOT, rather than all fibers reaching the IOT and subsequently becoming restricted to discrete patches of the optic tectum.

A further experiment in Type II fish injected with labeled proline into the contralateral eye revealed that the existing projection, which had not been surgically altered, had become discontinuous as a consequence of superinnervation. Fish injected with labeled proline into both eyes indicated that the entire remaining hemitegment was innervated in a discontinuous fashion by both eyes.

Visual testing of Type I fish following regeneration indicated that most animals responded to an overhead moving shadow by reducing their respiration. Fish that had a sparse IOT projection, on the basis of subsequent autoradiographic analysis, did not respond to the shadow. Type II fish responded equally well with either eye in the shadow test. However, an optomotor response could only be elicited from the contralateral, but not the ipsilateral eye. These results suggest that the IOT projection in Type II fish is only partially functional. Supported by NSF BNS-7815081.

**2330**


Axons regenerate rapidly in the dorsal root of the adult cat after an injury to the nerve several centimeters from the spinal cord. Regenerating axons are associated with Schwann cells in endoneurial portions of the nerve and eventually become remyelinated by them. However, the nerve fibers do not enter the spinal cord. They stop growing precisely at the root entry zone which represents the boundary between central and peripheral nervous tissue. Regenerating axons were not observed to penetrate the layer of astrocyte processes at the surface of the spinal cord in animals surviving up to two years. Myelinated and unmyelinated axons at the root entry zone end blindly. Many contain organelles characteristic of regenerating axons, some have terminal enlargements, but none give rise to the myelinated axons characteristic of a neuron. The fact that astrocytes block the regenerating nerve fibers even though the initial injury was situated some distance away from the root entry zone and did not produce a glial scar indicates that they possess growth inhibiting properties. The results of this investigation suggests that the mature astrocyte may constitute the principal impediment to axon regeneration in the central nervous system of mammalians.

**2331**


In recent publications we have reported pronounced effects elicited by NGF and its antiserum (anti-NGF) on the retinal ganglion cell body response to axotomy and optic nerve regeneration in the newt. In this abstract we wish to report that most animals responded to an overhead moving shadow by reducing their respiration. Fish that had a sparse IOT projection, on the basis of subsequent autoradiographic analysis, did not respond to the shadow. Type II fish responded equally well with either eye in the shadow test. However, an optomotor response could only be elicited from the contralateral, but not the ipsilateral eye. These results suggest that the IOT projection in Type II fish is only partially functional. Supported by NSF BNS-7815081.

**2332**

**INDUCED PLASTICITY IN VISUAL CORTEX OF ADULT CATS.** D. N. Spinelli, Frances E. Jensen* and Leanna Standish*, Department of Computer and Information Science and Psychology, University of Massachusetts, Amherst, MA 01003.

During the critical period of development (6 to 10 weeks) the visual cortex of kittens exhibits remarkable plasticity. kittens viewing vertical lines with one eye and horizontal lines with the other during this period lose binocular cells in visual cortex and exhibit instead neurons which are monocular and selective for lines of orientation similar to that viewed by the eye that activates them. These effects cannot be reproduced in adult cats, suggesting that plasticity and neuronal growth might be related. Colchicine has been shown to produce temporary chromatolysis, nucleolar enlargement, retraction of presynaptic terminals and loss of synaptic transmission when applied locally to neural tissue. These effects are reversible in 3 to 4 weeks. We wanted to investigate the possibility that during recovery plasticity might be present. To this end, a solution of colchicine (2µl mole) was applied to the visual cortex of one hemisphere in adult cats for 30 minutes. After recovery from surgery the cats were exposed to goggles, with vertical lines for one eye and horizontal lines for the other, 4 hours every day for a period of 2 months. The animals were kept in a dark room at all other times. At the end of two months, single cells were recorded from the visual cortex of the colchicine-treated hemisphere and from the untreated one, which served as control, and their receptive fields were mapped. The data shows that extensive reorganization of receptive field orientation, sensitivity and binocularity took place in the colchicine-treated hemisphere. Here, cells were mostly monocular and responded to bars of the same orientation viewed by the driving eye during recovery. A few cells were found that responded to vertical lines when tested through the eye that had viewed horizontal lines, and also to horizontal lines when tested through the eye that had viewed horizontal lines. These cells were never found normally. Cells in visual cortex of the untreated hemisphere exhibited normal properties and receptive field types, i.e., they were mostly binocularly driven, and were distributed evenly in terms of orientation sensitivity. Histological analysis indicates that some neuronal rearrangement had taken place in the treated cortex. The results will be discussed in terms of the interaction between experience and the induced neuronal modifications.

**2333**

**REGENERATION**

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The implants became vascularized rapidly. The original axons of the graft had undergone Wallerian degeneration, and the myelin debris was degraded by graft Schwann cells and hematogenously-derived macrophages. At three weeks, there was clear evidence by EH of regenerating axons throughout the graft in association with Schwann cells. Elongated Schwann cell processes encircled individual axons or groups of axons.

At six weeks, the regenerated axons in the graft had PNS myelin around them, distinguishable from CNS myelin by its periodicity and the presence of a basal lamina. Several groups of remyelinated axons were fasciculated in the pattern of a regenerating peripheral nerve.

In the CNS parenchyma bordering the implant, myelination of the regenerating CNS axons was observed. Along most of the border of the implant, the laminae were comprised of recently-proliferated oligodendroglial cells and thinly-myelinated CNS axons presumably en route to the transplanted tissue.

The findings of this study are consistent with the concept that transplanted myelinating cells may produce soluble factors which are attractive to regenerating axons.


The thyroid hormones triiodothyronine (T3) and L-thyroxine have been shown to enhance regeneration of peripheral nerves 

Previous studies from this laboratory (Brain Res., 138, 423, 1977) have shown that rat iris tissue transplanted into the autologous rat midbrain will be myelinated by CNS axons, some of which have PNS myelin formed by graft Schwann cells. The present experiment follows a similar protocol. Segments of rat peripheral nerve were transplanted in the midbrain. Animals were perfused and examined under sterile conditions, thial nerve grafts 3mm in length were placed through a burr hole in the cranium into the midbrain at the edge of the periaqueductal gray matter, in a rostral-caudal position. The overlying skin was sutured. Animals remained healthy and showed no clinical abnormalities. Animals were perfused at 3 weeks and 6 weeks post-implantation and the midbrain tissue was prepared for morphological study.

The myelin sheath myelin sheath thickness increased from a control value of $65 \pm 5\%$ to $85 \pm 9\%$ at six weeks. The myelin sheath thickness increased from a control value of $65 \pm 5\%$ to $85 \pm 9\%$ at six weeks. Both voltage and current threshold decreased slightly from a control value of $-49 mV \pm .59$ at four to six weeks, and then increased to $-56 mV \pm 1.15$ by eleven weeks. Both voltage and current threshold decreased slightly from a control value of $-49 mV \pm .59$ at four to six weeks, and then increased to $-56 mV \pm 1.15$ by eleven weeks.

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2336 SELECTIVE REGENERATION OF OPTIC NERVE FIBERS INTO THE OPTIC TECTUM AFTER TECTAL REIMPLANTATION OR TRANSPLANTATION IN ADULT GOLDFISH. Myong G. Yoon and F. A. Baker*. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada.

In normal goldfish, a majority of optic fibers invade the tectum via the stratum opticum, and terminate at the stratum fibrosum et griseum superficiale in a consistent topographic pattern. In the operated tectum, the passages of regenerating optic fibers within the altered cytoarchitecture of the surgically-operated tectum are examined after reimplantation of the tectal tissue, or after tectal resection. These changes are maximal at about three weeks and were seen in progressively fewer cells at increasing distance from the transaction site. These changes included: 1) loss of most cytoplasmic staining and concentration of chromophoric substance in a thin ring around the nucleus, 2) an increase in the proportion of cells with eccentric nuclei (defined as nucleus contacting the outer cell margin), 3) an increase in nuclear size and a decrease in cell diameter with a consequent striking increase in nuclear/cell diameter ratio from 34 to 41 in the first 5 mm caudal to the transaction, and 4) an abrupt concave down trend in stratification after reimplantation of the tectal tissue. These changes are consistent with the concept that transplanted myelinating cells may produce soluble factors which are attractive to regenerating axons.
SENSE ORGANS
of these showed an increase in firing activity as the frequency of vibration was increased. The majority (69%) of the responsive neurons to extraocular muscle vibration showed tonic responses. The vibratory afferents in these muscles have been examined in spindles of biventer cervicis and complexus using an ordered battery of staining techniques: myofibrillar ATPase (mATPase); mATPase subsequent to alkali preincubation; mATPase subsequent to acid preincubation. These techniques differentiate the nuclear bag fibres of two-bag spindles into 2 types in a manner consistent with descriptions of spindles in cat hindlimb. One of the bag fibres (bag1) reacts intensely for mATPase activity following alkali preincubation but shows only light staining following alkali preincubation. The second fibre (bag2) stains intensely following alkali preincubation and with moderate intensity following acid preincubation. By comparing the staining profiles in one-bag spindles with the profiles of two-bag spindles, the type of bag fibre present in one-bag spindles can be determined. In all one-bag spindles which have been so far reconstructed in complexus, the nuclear bag fibre which is present has a staining profile consistent with that of a conventional bag2 fibre. Whether the loss of bag1 fibres in these spindles has a major effect on the encoding properties of the primary ending remains to be determined.

Supported by the M.R.C. of Canada.

PERIPHERAL AND CENTRAL EFFECTS OF TONIC VIBRATORY STIMULI TO DORSAL NECK AND EXTRAOCULAR MUSCLES IN THE CAT. H. Barbas and H. B. is now at Harvard Neurological Unit, Beth Israel Hospital, Boston, MA.

Sensation of chloroform anesthetized cats at latencies of 5-50 ms (Exp. Neuros. 9: 600-609, 1972; Neuros. Abstr. 11: 466, 1977). They now report that neurons in these regions, which correspond with the frontal eye fields, also respond to vibratory stimuli (25-330 Hz, 20-200 µm) applied to extraocular and dorsal neck muscles. The observed evoked neuronal responses occurred 10-90 ms following the onset of vibration, and were characterized either by a phasic burst of cellular activity, or tonic responses which lasted for the duration of stimulation. Neurons could be further classified into those whose response depended on the frequency of vibration, and those that did not. In 12 cats 37 neurons showed consistent responses to vibration of either the biventer cervicis or the rectus capitis dorsalis major muscles of the dorsal neck. Of these nine showed an increase in firing activity as the frequency of vibration was increased (100-330 Hz) at amplitude displacements of 20-50 µm. Intravenous injection of the depolarizing neuromuscular blocking agent succinylcholine (30-40 µg/kg), known to activate preferentially the primary endings of muscle spindles, also activated these neurons. In nine cats 39 neurons responded to vibration of the superior rectus muscle of the eye at stimulation rates of 20-160 µm, and 25-300 Hz frequencies, or 0.5-1.5 mm single stretches of the muscle. Consistent increases in firing activity with increases in vibration frequencies (100-330 Hz) were observed in seven of these neurons, including three which showed increased responses to faster single pulls of the muscle. The majority (95%) of the responsive neurons to extraocular muscle vibration showed tonic responses to 50% of the maximum supramaximal stimulus, suggesting that these neurons are active in the primary sensory rosette, rather than in the second order neurons to the eye, which was abolished with administration of the paralytic agent gallamine triethiodide. This result demonstrated that a tonic stretch reflex can be elicited in both dorsal and extraocular muscles with vibratory stimuli, suggesting the presence of a proprioceptive feedback control system in both of these muscle structures. The neural responses to low threshold afferents originating in muscle receptors further suggests that small head and eye movements can activate these frontal brain regions, which may participate in mechanisms underlying coordinated eye and head movement.


To determine the susceptibility of the squirrel monkey to motion sickness, combined horizontal rotation and vertical motion tests were carried out to determine the specific effect of 10, 25 and 50 r.p.m. rotational rates with a vertical excursion of 2 g net acceleration with otoconial weights from both stationary embryos and embryos rotated at the center of the centrifuge grown at 1 g. Under these conditions the mean otoconial weight (n=56) of the experimental animals was 1.62% lighter than that of the stationary controls (n=35, p<0.05, 1 tailed T test) and 14.7% lighter than that of the rotated controls (n=47, p<0.0005, 1 tailed T test).

Scanning electron micrographs were taken of the utricular otoletic organ and control embryos of 25 days of age. The otolithic organ of a 29 day old control embryo was examined in spindles of biventer cervicis with and without application of a tracer dye. The histochemical profiles of nuclear bag fibres have been examined in spindles of biventer cervicis and complexus using an ordered battery of staining techniques: myofibrillar ATPase (mATPase); mATPase subsequent to alkali preincubation; mATPase subsequent to acid preincubation. These techniques differentiate the nuclear bag fibres of two-bag spindles into 2 types in a manner consistent with descriptions of spindles in cat hindlimb. One of the bag fibres (bag1) reacts intensely for mATPase activity following acid preincubation but shows only light staining following alkali preincubation. The second fibre (bag2) stains intensely following alkali preincubation and with moderate intensity following acid preincubation. By comparing the staining profiles in one-bag spindles with the profiles of two-bag spindles, the type of bag fibre present in one-bag spindles can be determined. In all one-bag spindles which have been so far reconstructed in complexus, the nuclear bag fibre which is present has a staining profile consistent with that of a conventional bag2 fibre. Whether the loss of bag1 fibres in these spindles has a major effect on the encoding properties of the primary ending remains to be determined.

Supported by the M.R.C. of Canada.
SENSE ORGANS

FREQUENCY RESPONSE OF DEITERS’ NEURONS TO NATURAL STIMULATION OF NECK AND MACULAR LABYRINTHINE RECEPTORS. R. Boyle* and C. Pompeiano.

University of California, Los Angeles, CA 90024.

The frequency responses of Deiters’ (UV) neurons to stimulation of neck and labyrinthine receptors has been studied in precocious, decerebrate cats during sinusoidal rotation. The peak amplitude at 0.026 Hz: a) of the cervical axis while maintaining the head horizontally (neck input), b) of the whole animal in both directions (the median plane (labyrinth input) of Deiters’ nucleus) and c) of both the head and neck while maintaining the body horizontally (neck and labyrinth input). Seventy out of 100 units (58.3%) tested during neck stimulation and 77 out of 102 units (75.5%) tested during labyrinth stimulation showed a periodic modulation of the firing rate in response to the sinusoidal input, the phase angle being mainly related to the extreme neck or head position. The average sensitivity of the responses, expressed in per cent increase of the mean firing rate per degree of displacement, was higher for the neck (2.5 ± 1.7%, S.D.) than for the macular input (2.1 ± 2.1%, S.D.). Among the units responsive to the neck input, 76/100 (i.e., 76%) were located in the rostroventral LCN (CRF) (sensitivity: 3.84 ± 7.54%, S.D.), while 36/76 (i.e., 48%) were located in the deccerebral LCN (CRF) (sensitivity: 2.75 ± 2.54, S.D.). Among units responsive to the labyrinth input, 67/100 (i.e., 69.0%) were located in the CN (sensitivity: 1.72 ± 1.80, S.D.). It appears therefore that the sensitivity to the neck and labyrinth inputs is greater in the forebend CRF than in the hindlimb LCN region of Deiters’ nucleus.

Among 100 units tested to neck and labyrinth stimulation, 52 responded to both inputs and were mainly position sensitive, most of these units (73.5%) were excited during side-down tilt of the animal, while fewer units (46%) were excited during side-down modulation of the neck. Duration of the head alone, leading to combined stimulation of neck and macular receptors, yielded a response whose sensitivity and phase angle were significantly correlated with the expected vectorial values derived from the analysis of the individual responses. Due to the significant convergence and the stable interaction of neck and macular inputs, the CRF represents an important preeminent structure for the integration of the two inputs during the cervical and labyrinthine control of posture.


Department of Anatomy and Brain Research Institute, University of California, Los Angeles, CA 90024.

Fast-adapting stretch receptors in the posterior duct of a pigeon were labelled with horseradish peroxidase (PAM) of crayfish were submitted to sinusoidal length modulations with depths of 0.90 to 7.5 mm and frequencies of 2.1, 1.0, 3.0 and 10.0 cps.

Amounts of 0.5 µl of 50% HRP were delivered through a microsurgery catheter inserted into the cranial cavity. HRP was reacted by the tetramethylbenzidine (TMB) blue reaction process. Schwartz et al (Brain Res. 155:103-107, 1978) injected HRP into the lateral recessus of the cranial cavity and reported a bilateral cluster of DAB-labelled vestibular efferent neurons in the nucleus reticularis pontis caudalis. Using the techniques described above, we have extended these findings by identifying four distinct ipsilateral and two distinct contralateral groups of labelled vestibular efferent neurons.

Within the reticular formation the labelled, large multipolar efferent neurons were distributed equally bilaterally in all 5 pigeons (mean ± SD of cells per each side: 49 ± 25). The majority of these cells were clustered close together in the nucleus reticularis pontis caudalis immediately ventro-latero-caudal to the abducens nucleus. A separate, smaller cluster of labelled, similar sized, multipolar neurons was also noted within the same nucleus, adjacent and medial to the nucleus paragigantocellularis lateralis and ventro-latero-caudal to the first group of labelled cells.

Within the ipsilateral vestibular nuclear complex, labelled efferent neurons (mean ± SD of cells: 77 ± 25) were noted to cluster in distinct groups in 3 of 5 pigeons. A group of medium sized bipolar labelled neurons were located within the tangential nucleus, adjacent to and intermingled with, larger multipolar labelled neurons in the latero-caudal part of the inferior (descending) vestibular nucleus. A second group of large multi­ polar, labelled neurons (Deiter’s cells) interspersed with label­ ed medium-sized round and triangular cells were noted to cluster in the medial pole of the lateral vestibular nucleus adjacent to the nucleus laminaris.

A further group of small, labeled neurons were also noted in the ipsilateral nucleus laminaris and may represent cochlear efferent neurons. (Supported in part by the Deafness Research Foundation and NASA Contract NAS 9-14641.)
Cupula displacements were measured in the semicircular canal of the skate, Raja erinacea, under known loads. The cupula stiffness coefficient—the ratio of applied torque to angular displacement—was thereby determined. Procedures were carried out in artificial skate perilymph. The cupula was carefully avoided. Contact with the cupula at both the cristal and at the end of the ampulla base was assessed using Nomarski interference contrast optics. Preparations which by these criteria appeared unremarked were studied further under the Nomarski microscope. Small pieces of aluminum foil were placed upon the cupula and resulting cupula displacements at one or more points were measured. Displacements were in the range of 0.0005 to 0.0015 mm rad. The ratio of torque to displacement was thereby determined to estimate the physiological upper limit (3-5 micrometers) in the same species. Displacements at different points were consistent with the notion that the cupula moves as a unit about an attachment region at the cristal. The location of the foil allowed estimation of the torque about the cristal and thereby determination of the stiffness coefficient of the cupula. In some cases 2-4 pieces of foil were placed upon the cupula and thereby successive increments of displacement were measured; the stiffness coefficient increased as additional load was placed on the cupula. Measured stiffness coefficients were between 4 x 10^{-4} and 4 x 10^{-3} dyne cm rad in different preparations. These values are consistent with those that have been inferred in other species by less direct means. (Supported by NIH Grant NS11080).

In man, when retinal information is coupled to head motion, suppression of the vestibulo-ocular reflex (VOR) is marked at low frequencies of oscillation (e.g., gain of 0.1 at 0.1 - 0.5 Hz). In the absence of retinal information, image of the coupled visual signal yields a strong suppression of VOR gain. In darkness, an image of the visual signal, VOR gain and fast phase saccades are suppressed to a level equivalent to the response observed with retinal information. This magnitude of suppression persists without further training for a considerable period of time (at least 12 months). Attempts to detach the subjective by cognitive tasks do not lead to marked alteration in VOR suppression.

Phase advance of eye displacement relative to head displacement, observed with retinal information, is modified under all test conditions in the dark to an in-phase response. The results suggest that under these test conditions: 1) non-visual control of VOR suppression can be achieved by matching a "sense of eye movement or fixation" to the retinal image, 2) gain and phase control of the VOR may function independently.

REGIONAL INNERVATION DIFFERENCES IN THE UTRICLE OF THE GOLDFISH SENSE ORGANS

Sensory activity of different VIIIth nerve semicircular canal fibers shows a wide range of mean (µ), standard deviation (σ) and coefficient of variation (CV = µ/σ) of interspike intervals. We investigated whether these parameters were correlated with spatial position of fibers in the guitarfish horizontal ampullary nerve (HAN), shown previously to be comprised of 3 to 9 separated bundles (O'Leary, Dunn and Honrubia, Nature 256: 225-227, 1974) Epochs of 80 seconds of spontaneous activity from 285 afferent neurons were digitized on a PDP II computer system, with an interspike interval resolution of 100 microseconds, and edited to remove artifacts. Interspike interval histograms and parameters, µ, σ and CV were computed for each afferent. Each parameter was tested separately, using the Kruskal-Wallis test, to determine whether it was distributed uniformly among 7 different bundle areas of the HAN.

The results showed that both µ and CV were distributed differently in the nerve (p<0.0002), with greater values in "irregular" cells occurring in central HAN nerve bundles, and lesser values in "regular" cells occurring in extreme rostral and caudal HAN bundles. Differences in µ distributions were not significant at an acceptable level. The synaptic differential non-linearity of VIIIth nerve HAN projection patterns of the guitarfish HAN to the crista described by Dunn (J. Comp. Neurol., 183: 779, 1979), and with differences in HAN dynamic response characterstics.

The activity of 51 single afferent fibers innervating the horizontal semicircular canal of the bullfrog was examined during prolonged, horizontal-plane, angular velocity stimuli, both sinusoidal and triangular, having different amplitudes (0.5-5°/sec) and frequencies (0.01-3.3 Hz). During the prolonged stimulation of most cells, the peak neural response in successive cycles gradually declined over a 1-4 min. interval and thereafter remained essentially constant. The late, non-declining response was considered to be the steady state neural response, and attempts were made to fit it with a model composed of a linear element, corresponding to the static nonlinearity preceding, followed by a biased rectifier element. The model was inadequate since the slope of the x-intercept of the fitted biased rectifier was strongly dependent on stimulus magnitude, which would imply that neural sensitivity and threshold were input dependent. Moreover, it can be shown that such neural behavior cannot be explained by any model composed of an arbitrary linear system followed by an arbitrary static nonlinearity (e.g. power law, etc.).

Simulation studies based on this data suggest a new form of model for the rotational afferent pathway, which is consistent with findings of others. It is well known that an essentially static nonlinearity exists at the level of hair cell transduction. Furthermore, recent studies suggest that adaptation and high frequency lead are introduced proximal to this nonlinearity. Therefore, it appears that the rotational afferent pathway can be modelled, to a first approximation, by a linear Steinhausen element representing the hair cell transduction; a static, nonlinear element representing hair cell transduction; a linear, adaptation-like, high frequency lead element, possibly representing the hair cell pool; an afferent system and finally, a biased rectifier representing primary afferent action potential generation. This model could be approximately fitted to the steady state responses of 8 out of 12 suitably chosen cells, thus testing the hypothesis of complete independence of neural sensitivity and threshold on input amplitude.

Furthermore, the presence of the static nonlinearity preceding the adaptation element in the new model accounts for the decrease in neural response observed during prolonged stimulation, and appears to explain a number of previously reported nonlinear phenomena in several animals. Furthermore, the model supports the notion that nonlinear behavior is more commonly reported in cold-blooded than in warm-blooded animals because hair cells of warm-blooded animals are biased to normally operate in the more linear portions of their transduction characteristic.

(Support: Canadian NRC, Quebec Ministry of Education.)
SLEEP
ASSAYS OF MONOAMINE METABOLOIDS IN LUMBAR CSF SAMPLES FROM NORMAL HUMAN CONTROLS AND HYPERSOMNIAC PATIENTS. Kym F. Faull*, Chandrasekharan Nair*, Division of Neurological Surgery, Stanford University Medical Center, Stanford, CA 94305.

At the Stanford Sleep Disorders Clinic 35% of the patients who complained of Excessive Daytime Sleepiness (EDS) did not fulfill the criteria for narcolepsy or obstructive sleep apnea syndrome. In this study of 35 patients the EDS was the sole symptom and this was objectively confirmed by multiple sleep latency test scores. In an attempt to find a CNS neurochemical deficit characteristic of this disabling yet poorly-defined hypersomnolent condition, we collected lumbar CSF samples from 8 patients and 15 normal control subjects. At an interval of 24 hours, two lumbar punctures (LP) were performed on each individual; the second LP was performed after an oral probenecid dose. The samples were assayed by gas chromatography-mass fragmentography to determine the concentrations of the neutral and acidic monoamine metabolites HVA, DOPAC, HBG, and 5-HIAA. Results indicated that after the probenecid dose there was a significantly elevated concentration of HVA and 5-HIAA in the CSF samples taken from the patient population, suggesting increased turnover of dopamine and serotonin.


It is well established that electrical or chemical stimulation of the area postrema/nuc. tractus solitarius (AP/NTS) of the medulla produces EEG cortical synchronization and/or sleep. Recently, an anatomical projection from the AP to the nucleus raphe dorsalis was identified in the rat using the horseradish peroxidase method (Brain Res. 1977, 122: 229-242). Because of the involvement of the anterior raphe and AP/NTS in sleep mechanisms and the indication of an anatomical projection to the raphe from the AP/NTS we investigated whether stimulation of the region of the AP/NTS would influence the activity of single units of the anterior raphe nuclei.

Fourteen adult male rats were anesthetized with urethane, 1000 mg/kg, and had the dorsal medulla exposed for positioning of stimulating electrodes. Results from 44 raphe units showed that in these acute preparations stimulation of the AP/NTS at 0.1-0.3 mA, 1 Hz or 10 Hz (occasionally 1.0 mA was used) was without marked effects. Approximately 50-60% of the units showed no change in firing rates, 30-40% exhibited temporal suppression of firing, and 10-20% were excited during the stimulation. For all units the average duration of suppression was 57 and 16 msec at 1 Hz and 10 Hz, respectively. The magnitude of the suppression was only about 1/3 to 1/2 that previously observed after stimulation of the lateral habenula or substantia nigra (Stern et al., 1979, Expil. Neurol. and Brain Res. Bull. In press). Also, in several instances there was a complete dissociation between changes in raphe unit activity and cortical synchronization or desynchronization. Cortical synchronization was often observed to occur without changes in raphe unit activity, and in some cases raphe unit activity was altered without a change in cortical EEG waves.

In the present data suggest that in the rat there is not a strong influence of AP/NTS region on anterior raphe units, and that the cortical EEG synchronization produced by electrical stimulation of the AP/NTS is not mediated by changes in unit activity in the anterior raphe. (Supported by NSF grant ENG 77-04527.)

THE EFFECT OF AN EXPANDING EPIDURAL MASS ON THE INTRACRANIAL PRESSURE PROFILE IN THE SLEEPING MONKEY. Gü indüz Gücer and Ruth R. Leaverton. NCSU/NC State University, Department of Neurosurgery, 1979.

In summary, the present data suggest that in the rat there is not a strong influence of AP/NTS region on anterior raphe units, and that the cortical EEG synchronization produced by electrical stimulation of the AP/NTS is not mediated by changes in unit activity in the anterior raphe. (Supported by NSF grant ENG 77-04527.)
Several lines of evidence from our laboratory have suggested that macromolecules may participate in the regulation of REM sleep (Drucker-Colín et al. Exp. Neurol. 45, 1979). The experiments to be described below provide a novel approach to the study of sleep, and one that provides a more direct corroboration of the role of macromolecules. Sixty cats were used in this study half of those cats were perfused with a pull-push cannula in the midbrain reticular formation (MFR). The perfusates were dialyzed, lyophilized and resuspended in Ringer. This material was then injected into rabbits and antibodies obtained by standard procedures. These antibodies were purified in a DEAE-dextran column and were then concentrated in a millipore filter (45 um). These antibodies were then injected into the MFR at 10,50,100,500, and 1000 ug/50 ul doses. The effects of these antibodies on the sleep-wake cycle was compared to Ringer pre-immune serum, antibodies to cat serum, antibodies neutralized with whole antigen, or partial antigen from serum. All concentrations were identical. The results these experiments showed that the 100,500 and 1000 ug dose of antibodies to MFR proteins decreased REM sleep by 70% without affecting slow wave sleep (SWS). However, the two highest doses affected SWS transiently through an increase in its latency. This decrease in REM sleep came about through a decrease in the frequency but not in duration of REM periods. In contrast to normal cats, it was observed that this antibody decreased the phasic events of REM sleep, i.e. eye movements and multiple unit activity much in the same way as protein synthesis inhibitors do. Since antibodies neutralized with whole antigen did not affect sleep, it was suggested that antibodies neutralization with cat serum antigen did, it can be suggested that a special class of brain proteins seem to be involved in the triggering mechanisms of REM sleep.

A principal peak was noted at 1 cycle/hr. While coupling at this frequency was present as early as the first week of life for some pairs of variables, it did not become prominent in other pairs until 3 months of life. Differences in coupling between pairs of variables were observed between the normal group and SIDS risk group. These included decreased coupling between pairs of physiological variables with frequencies higher than 1 cycle/hr in the SIDS group, reflecting a difference in organization of sleep states between normal and risk infants.

In one case, 20 mg of TRH, v.o., was given for seven days and in the other case for eleven days. Both cases reported alleviation of Excessive Day-Time Sleepiness and associated symptoms, in contrast to normals, both subjects showed increase in Delta sleep. In one case with early onset REM, the latency was shifted to a normal or longer period with a return to early onset at the cessation of TRH. The other case showed one early onset. REM after beginning TRH. It is proposed that TRH is acting as a Neuroregulator in a balancing fashion. Further, that TRH may be useful in both the diagnosis and treatment of Narcolepsy.

Sleep cycle circadian rhythmicity following either enriched or impoverished environmental rearing is examined in this study. Mice reared in either super-enriched (SEE), regular enriched (REE), social control (SC), or isolate environment (IE) for 30 days. SEE and KEK groups show a general increase in total slow-wave sleep (SWS) in the 24 hr. cycle but the number of SWS episodes, mean duration of SWS episodes, and percent SWS of total sleep time (TST) is not significantly different from the SC group for the day cycle (0700-1900). Enriched rearing produces a significant and selective increase in the number of PS (Paradoxical Sleep or REM sleep) episodes, mean duration of PS episodes, total amount of PS time, and percent PS/TST throughout the 24 hr. cycle. IE reared mice also show a general increase in SWS primarily during the day with significant reductions in all measures of PS occurring exclusively in the day cycle. It is suggested that alterations of chronobiological sleep rhythms after differential rearing may be mediated by the interaction of the suprachiasmatic nucleus (SCN) with the Gigantocellular Tegmental Field (FTG) and locus coeruleus (LC). Our results provide additional support for the hypothesis that PS occurring over a protracted time period is a requisite neurobiological mechanism for the processing, maintenance, and storage of long-term memory.

Respiratory neurons of the pneumotaxic center during sleep and wakefulness.

Ralph Lydic and John Orten, Department of Physiology, Texas Tech University School of Medicine, Lubbock, Texas 79440.

Extracellular recordings, using tungsten microelectrodes, were made from the pneumotoxic center (PNC) of intact, anesthetized cats during sleep and wakefulness. Contrary to previous work using decerebrate cats (Feldman et al. Brain Res. 104: 341, 1976) the awake, intact cat demonstrated respiratory neuron activity which was strongly respiratory modulated. During wakefulness, phasic neuronal bursts with average frequencies up to 50 Hz were observed superimposed on a tonic background. There was a decline in the activity of these cells during sleep. This decreased activity began in NREM sleep and was more fully expressed in REM sleep where the discharge rate was only 30-40% of the wakefulness value. These results suggest a loss of respiratory drive to the PNC during sleep.

Supported by Tarbox Parkinson's Disease Institute, Texas Tech University School of Medicine, and Heart Lung and Blood Institute, Grant 5801 HL-21257-02.

Paradoxical sleep without atonia in cats is accompanied by excessive exploratory behavior in wakefulness.

Adrian R. Morrison, C'azenna Mann*, Joan Hendricks*, and Catherine Starkweather*. Laboratories of Anatomy, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 USA.

Paradoxical sleep without atonia is a dramatic phenomenon created by small bilateral lesions in the pons (centered at P2.1, L2.0, H-3.5, Berman²) in cats and is characterized as follows: After slow wave sleep, when paradoxical sleep with muscle atonia would normally appear, cats raise their heads, make body contractions, and scan the environment. These movements, exhibit alternating movements of the limbs, and involve the eyes to scan the environment. Throughout an episode, which shows all other aspects of paradoxical sleep, including unresponsiveness to visual stimuli, cats act as if they are being startled, searching and sometimes attacking an object. In wakefulness, they show minor cerebellar signs. We have postulated that these lesions disrupt pontine excitation of the modulatory inhibitory area and inhibitory of a brainstem locomotor center during paradoxical sleep.

In six cats we tested whether these sleep effects were transferred in some way to wakefulness. All exhibited in wakefulness an increase of 23-127% in exploratory locomotor activity, which consisted of moving about a room marked into squares and investigating various parts of the room rather than aimless pacing. This behavior was measured 1 hour/day for 5 days pre- and postoperatively.

The existence of a parallel effect in wakefulness and paradoxical sleep produced by pontine lesions supports our hypothesis that events of normal paradoxical sleep, which is a hyperalert brain state, are the result of exaggeration of a brainstem mechanism designed to dampen responses to sudden, novel stimuli in wakefulness in the animal over react and run blindly into danger prior to stimulus analysis. The results also suggest that paradoxical sleep without atonia is more than a simple abolition of the cerebral inhibition of spinal motor neurons which occurs in normal sleep and that the animals are not acting out normal "dreams".


Research supported by NIH Grant NS-13110.
MEDULLARY RESPIRATORY NEURON ACTIVITY: RELATIONSHIP TO TONIC AND PHASIC REM SLEEP

John M. Orem, Ph.D., Department of Physiology, Texas Tech University School of Medicine, Lubbock, Texas 79430.

This study analyzed the relationship of brain stem respiratory neuron activity to the tonic and phasic events of rapid eye movement (REM) sleep. Dorsal and ventral medullary respiratory neurons were recorded in sleeping cats. Discharges of inspiratory and expiratory cells increased in number and average frequency with increases in ponto-geniculo-occipital (PGO) spiking (phasic REM activity). The correlations between PGO wave frequency and respiratory neuron activity were positively related to the discharge levels of the neurons: the more active the cell, the greater the relationship to PGO activity. Tonic REM influences on respiratory neurons were calculated by extrapolating from the regression line relating PGO frequency and neuron activity to the hypothetical state of no PGO activity. These calculated levels, when compared to non-REM sleep levels, showed that tonic REM mechanisms recruited some neurons and activated others.

Decruited cells tended to be found in the ventral medullary respiratory group; activated cells were generally in the dorsal group. These results demonstrated a respiratory activity to non-respiratory REM sleep variables.

Supported by Heart, Lung and Blood Institute, Grant 5861 - HL-21577-02.

EFFECT OF SLEEP ON BENZODIAZEPINE BINDING IN BRAIN.

M. E. Poddar, D. A. Englhart and A. K. Sinha. Department of Physiology & Biophysics, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, N.J. 08854, USA.

In vitro specific [3H]-diazepam binding was determined in the cerebral cortex (C), upper brain stem (UBS), cerebellum (Cr) and pons-medulla (P-M) of adult, male, hamsters (body wt. 100-120 g) after sleep and wakefulness. Animals were decapitated following 20 or 50 min. of non-rapid-eye-movement sleep or a comparable period of wakefulness. Immediately after sleep the specific binding of diazepam increased in all four regions of brain in contrast to liver, kidney and lung were no changes in specific binding of [3H]-diazepam were detected between sleep and wakefulness. This increased diazepam binding was found to be dependent on length of the sleeping time.

Sleeping time % increase of [3H]-diazepam binding in brain regions (min)

<table>
<thead>
<tr>
<th>Region</th>
<th>20</th>
<th>30</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>36</td>
<td>64</td>
<td>107</td>
</tr>
<tr>
<td>UBS</td>
<td>21</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>Cr</td>
<td>21</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>P-M</td>
<td>34</td>
<td>34</td>
<td>87</td>
</tr>
</tbody>
</table>

The increased diazepam binding in brain regions after sleep may be due to (1) a change in the number of binding sites (Bmax) and/or (2) a change in the binding affinity (1/KD). Scatchard plot analysis of diazepam binding in cerebral cortex, upper brain stem and pons-medulla showed a significant increase in the affinity of the receptor for the ligand after sleep whereas no significant change was found in the number of binding sites between sleep and wakefulness.

Brain % increase (with respect to awake) after 50 min sleep

<table>
<thead>
<tr>
<th>Region</th>
<th>Bmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>37.7 (p&lt;0.01)</td>
</tr>
<tr>
<td>UBS</td>
<td>20.3 (p&lt;0.05)</td>
</tr>
<tr>
<td>Cr</td>
<td>20.3 (p&lt;0.05)</td>
</tr>
<tr>
<td>P-M</td>
<td>30.47 (p&lt;0.05)</td>
</tr>
</tbody>
</table>

This increased diazepam binding affinity in brain regions after sleep suggested that there may be a possibility of an increase in concentration of gamma-aminobutyric acid and/or a decrease in concentration of diazepam binding inhibitors, including any possible endogenous ligand for the benzodiazepine receptor. Studies on cross mixing experiments of diazepam binding with synaptic membrane in presence of 30,000 g supernatant of sleeping and awake brain regions indicate the presence of a diazepam binding inhibitor in awake brain. Further studies are now in progress to characterize the inhibitor present in the 30,000 g supernatant of the awake animal's brain.

SLEEP

NUCLEAR MUSCLE TONUS DURING SLEEP, WAKEFULNESS AND TONIC IMMOBILITY IN THE RABBIT. R. T. Pivik, P. Sussman and C. Braun. Dept. of Psychiatry University of Ottawa, School of Medicine, Faculty of Health Sciences, School of Psychology, University of Ottawa.

A cardinal characteristic of paradoxical sleep (PS) is a suppression of nuchal or facial muscle tonus. Tonic immobility (TI), a reversible state of immobility induced by rapid dorsoflexion and characterized by loss of light reflexes and relaxation of the masticatory muscles, was reported to be due to the absence of nuchal musculature and spinal reflexes similar to that occurring during PS. Previous reports examining muscle tonus in the rabbit have been descriptive and have not provided quantitative intra- or inter-state comparisons. In the present study nuclear EMG activity was quantified by means of resetting integrators and comparisons made between states of sleep, wakefulness and tonic immobility.

Seven (7) New Zealand White adult male rabbits were chronically implanted for recording EEG, nuchal EMG (telfon coated stranded stainless steel wire) and EOG activity. Recordings began one week after surgery. Spontaneous sleep-waking cycles were recorded in unrestrained animals in a light, sound and temperature controlled environment. TI was induced by rapidly inverting the animals in a "V" shaped trough. TI data were gathered in the late afternoon or early evening after sleep recordings. EMG activity was categorized according to the following conditions: active wakefulness (AW), quiet wakefulness (QW), transition to sleep (drowsy, D), slow wave sleep (SW), PS and TI. The transition from SW to TI was also examined. Statistical comparison among states was conducted using analyses of variance with post-hoc t tests when appropriate.

Muscle activity progressively decreased from AW through states QW, D, SW and PS. EMG activity during PS was least of all conditions, but differed significantly (p < .001) only from AW. Muscle activity decreased during the transition (1 minute from SW to PS), but the reduction during TI relative to the immediately preceding SW epoch was not statistically significant. Tonic muscle activity during TI was at a level more similar to SW than PS.

Tonic levels of nuchal EMG activity during PS and TI were quite variable. In our experience, neither state is consistently characterized by tonic activity.

EFFECT OF ALCOHOL ON SLEEP AND NIGHT-TIME PLASMA GROWTH HORMONE AND CORTISOL LEVELS.

T. A. Roehrs, P. N. Printz, E. D. Weitzman and M. Linnoila* (SPON: W. Dong). Univ. of Wash., Seattle, WA 98195, Montefiore Hospital, Bronx, NY 10467 and Duke Univ., Durham, NC 27710

The acute and chronic effects of alcohol and alcohol withdrawal on sleep patterns and on growth hormone and cortisol fluctuations occurring during sleep were studied. Before going to bed five healthy men, aged 21–26, received a placebo drink for three baseline nights, alcohol (1g/kg)-for eight nights, and no drink on a final withdrawal night. After two adaptation nights in the laboratory, standard all night polygraphy sleep recordings and blood samples, obtained every 20 minutes through indwelling venous catheters, were collected starting one night each in the baseline, acute (alcohol) night 1, chronic (alcohol) night 8, and alcohol withdrawal conditions.

On both drug nights, blood alcohol levels peaked within an hour of bedtime at 80 mg percent and the effect of alcohol on sleep stage percentages was confined to the first half of the night. Acute and chronic alcohol significantly reduced REM sleep. Slow wave sleep (stages 3 and 4) was increased significantly after acute alcohol, but returned to baseline levels on the chronic alcohol night. On the withdrawal night there was a slight, but non significant, increase in REM sleep and no change in slow wave sleep relative to baseline levels. The other sleep stage measures were not altered significantly by alcohol administration and withdrawal.

Alcohol, on both the acute and chronic nights, significantly suppressed (by 70%) plasma growth hormone levels across the whole night. All measures of the growth hormone response, total for the night, hourly rate, and peak level, were affected similarly by alcohol. There was no rebound in growth hormone levels on the alcohol withdrawal night. Night-time plasma cortisol levels were not affected by acute and chronic administration and withdrawal of alcohol.

The theoretical and clinical implications of the alcohol effects on sleep patterns and on growth hormone release will be discussed.

Five male hamsters (120 g) were sleep deprived for 24 hr by manual or mechanical manipulation. Their brains were fixed in situ by perfusing a mixture of glutaraldehyde and formaldehyde through the heart. Control animals were prepared identically. Pieces of frontal cortex, occipital cortex, and liver were treated with osmium tetroxide, dehydrated and embedded. This sections were examined by electron microscope. Stereological analysis was performed by taking low power (5,600 x) pictures of randomly encountered neurons and astroglial cells in five different areas of the frontal cortex of both the control and experimental animals. The partial volumes, relative surface areas and relative number of subcellular components were determined in the micrographs projected on a standard morphometric test-grid. The cell-bodies of neurons from the IVth and Vth layers of frontal and occipital cortex were scanned for structural alterations. Nuclear membrane, chromatin distribution pattern, mitochondria, Golgi apparatus, and cell membrane show no changes. The partial volumes of endoplasmic reticular cisternae and the surface area of the endoplasmic reticulum remained constant in the neurons of sleep deprived animals. Also, the number of neuronal lysosomes decreased by 46% in frontal cortex. No changes in the liver cells were detected. The astroglial cells of the sleep deprived animals were found to contain large clear vacuoles. In 80% of the sleep deprived astroglial cells the endoplasmic reticulum vesiculated with partial desolution of its membrane as compared to only 14% in the control animals. There is no change in the surface area of endoplasmic reticulum. The volumes of astroglial endoplasmic reticular cisternae increased by 162% in frontal cortex and 122% in occipital cortex of the sleep deprived animals. The fact that the surface area of the endoplasmic reticulum remains the same but the volume increases gives support to the idea that the membrane bound spaces seen in the astroglial cytoplasm are vesiculation of the endoplasmic reticulum rather than its new formation. Incapacitating behavioral decrement associated with sleep loss may be a consequence and symptomatic expression of the type of subcellular disorganization observed in this study. (Supported by grant #78-3532A from Air Force Office of Scientific Research).

INTERSPECIES COMMONALITIES IN MOTILITY PATTERNS DURING SLEEP AND WAKEFULNESS. Lorna B. Thomas, Dept. of Biobehavioral Sciences, University of Conn., Storrs, Ct.06268.

Motility recordings of infant and adult rats and rabbits, and of brown rats from birth to six months of age were used to demonstrate commonality in motor expression of sleep and waking states. The analog output from a motility sensor was produced by bypassing the LGN and marginal gyrus following the method of Singer (Brain Res., 35:55, 1973). The occipital PGO (recorded from the marginal gyrus) was compared to both: (1) the PGO recorded from the pons and the LGN, and (2) the "directly" transmitted PGO waves recorded from the marginal gyrus of cats either after surgical removal of the LGN, or transsection of the geniculo-cortical pathways. Two methods were used to study the effects of non-visual stimuli on the geniculate and the two types of occipital PGO waves: (1) electrical stimulation of the medial part of the ventral LGN, and (2) non-visual sensory stimulation (clicks, skin of foot, etc.) administered in presence and absence of reserpine. The results suggest that a potential mechanism for encoding PGO waves consists of vestibular activation of specific neurons of the pontine paramedian tegmentum under the influence of a specific set of pontine pacemaker cells. The occipital PGO waves of intact animals result from combination of the geniculate and the "direct" occipital PGO waves (a cortical activity under the direct influence of the pontine PGO that ascended by bypassing the LGN). The differences between the "direct" occipital PGO and those of intact cats are statistically significant and consist of the presence of a first positive going spike and greater differences in shape and amplitude of the occipital waves found in the intact cat. Mathematical treatment of the data suggested that the occipital PGO is a vectorial sum of the geniculate and "direct" occipital PGO waves. The first positive going spike seems to be related to the activity of the infrageniculate terminal of the optic tract as changed in amplitude and shape by the activity of LGN. The PGO waves were affected by endogenous and exogenous non-visual sensory processes, more in the occipital cells alone.
SOMATIC SENSOR SYST S
DEGENERATION OF TRIGEMINAL PRIMARY SENSORY NEURONS AFTER SKIN REMOVAL. Jan Arvidsson* and Gunnar Grant. Department of Anatomy, Karolinska Institutet, S-10401, Stockholm, Sweden.

In previous studies it was shown that transaction of peripheral branches of trigeminal primary sensory neurons in adult rats and cats resulted in degeneration of the centrally projecting branches. Ultrastructural studies of this type of axonal degeneration, termed transganglionic degeneration, revealed alterations similar to those occurring during Wallerian degeneration. Studies of the trigeminal ganglion following peripheral nerve transaction showed a substantial cell loss at the light microscopic level, as well as ultrastructural signs of degenerating and dying nerve cell bodies. Since the transaction in those studies was made far out peripherally, the question was raised whether simply disconnecting the nerve cell body from its receptors might itself induce transganglionic degeneration. As a first approach small areas of skin were removed supraorbital (2 rats), infraorbital (2 rats) and in the mental region (2 rats). The edges of the incisions were apposed and sutured. After postoperative survivals of 14 or 24 days the rats were anesthetized and necropsied processed according to the Fink-Heimer technique. In all cases there were small amounts of degeneration in the rostral part of the spinal trigeminal nucleus and soma of primary sensory neurons was found in the other trigeminal nuclei. The location of the degeneration was strictly within the same dorso-ventral and medio-lateral areas as after supraorbital, infraorbital or mental nerve transection shown in previous studies. The results support the view that disconnection of the nerve cell body from the receptor area may be an important factor for provoking transganglionic degeneration in the central nervous system of adult animals following skin removal does not seem to have been shown before. It clearly demonstrates the possibility of using this approach to study the effects of removing the body surface on the primary sensory relay nuclei. Hopefully further studies will give an answer to the question whether this approach will be useful also for mapping the central representation of different types of receptors.

Supported by the Swedish Medical Research Council, Project No. 553.

Recent studies from this laboratory have described the organization of spinal projections from the medial posterior parietal cortex of area 5, termed the supplementary sensory area, and from the medial cortex of area 6 comprising the supplementary motor area in primates. Here we report the intracortical and thalamic connections of these two cortical regions determined by the retrograde horseradish peroxidase (HRP) and anterograde autoradiographic tracing methods.

Cortical HRP injections were made by multiple penetrations with 0.3-0.6 µl of 25-50% HRP solution. The tissue sections were then reacted with the tetramethylbenzidine technique. Anterograde studies were performed by injections of 0.03-0.07 µl (100 µCl/µl) of a mixture of tritiated proline and leucine into either cortical region followed by routine processing of the tissues for autoradiography.

The supplementary sensory area receives somatotopically organized projections from the posterior parts of SI (mainly areas 1 and 2), from the lateral part of area 5, from the lateral and medial parts of area 6 comprising the supplementary motor area. The supplementary motor area similarly receives somatotopically organized projections from the SI cortex (mainly areas 1 and 3), from the lateral part of area 5, from the primary (area 4) motor cortex (MI) and from the supplementary sensory area (medial area 5).

Thalamic afferents to the supplementary sensory area arise primarily from the dorsolateral part of the lateral posterior nucleus (Lp) which contains a large number of neurons being seen as far anterior as the caudal ventrolateral nucleus (VLo) and as far posterior as the caudal ventroposterolateral nucleus (VPLp). Thalamic afferents to the supplementary motor area arise from the lateral and dorsal parts of the ventral lateral nucleus (both VlC and VLo), extending forward into the ventral anterior nucleus (VAn) and caudally into the lateral-most part of the ventro-posterolateral nucleus (VPLp).

Both the thalamic, as well as intracortical pathways (with the exception of part of the SI projection) for the supplementary sensory area and supplementary motor area are reciprocal.

In view of the connections of these two cortical areas, the supplementary sensory area and supplementary motor area would appear to be capable of analyzing and programming complex sensory and motor behavior. (Supported by NS12481.)

ABSENCE OF ALTERATIONS OF DORSAL Horn SOMATOTOPY AFTER UNILATERAL L7 SECTION IN CAT. Paul B. Brown, J. Richard Kocher, and Robert F. Yeckel. Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Single units were recorded in laminas I-VI of the left dorsal horn of adult cats following a unilateral section of L7 dorsal root, using T2-epinephrinized, urethane-anesthetized animals. Light touch receptive fields were mapped on a standard cat leg with T2-epinephrinized micropipettes. Recording sites were marked by the Prussian Blue method.

Statistical analysis revealed no changes in the somatotopic organization. No significant differences in the size, shape, and overlap of receptive fields were identified. The number of responsive units was similar on the operated and control sides.

These findings support the hypothesis that the normal somatotopic organization of the spinal cord remains intact following a single dorsal root section.
Textured Surfaces Explored with the Moving Fingertips: Their Representation in the Discharge of Digital Nerve Fibers.

Ian Darian-Smith, Linda Oke*, Ian Davidson* and Kenneth Johnson.
Sensory Processes Laboratory, Department of Physiology, University of Melbourne, Parkville 3052, Australia.

Textured surfaces actively explored with the fingers, or moved across the stationary finger, are among the commonest stimuli that we identify and characterise tactually. Experiments were designed to examine the representation of the spatial and temporal features of two geometrically patterned surfaces moving across the skin (temporal frequency > spatial frequency x velocity) rather than the spatial features of the surface. The spatial characteristics of the surface could be represented only in the discharge pattern of a subset of more sensitive fibers, and in assemblies of these fibers. The stimulus used in these experiments applied the moving surface to the skin for a limited time with a fixed contact force and at a particular velocity. The surfaces used were fine gratings defined by their spatial frequency, and geometric arrays of elevated 'dots' defined by the dot diameter and the spacing of these dots.

Single mechanoreceptive fibers innervating the fingertip pad were isolated by dissection of the median nerve in an anesthetised monkey (Macaca nemestrina). Each fiber was then identified as a slowly adapting (SA), quickly adapting (QA), or Pacinian afferent.

Fibers responded to gratings moving across the fingertip with a very regular discharge which, however, reflected the temporal frequency (spatial frequency x velocity) rather than the spatial features of the surface. The spatial characteristics of the surface could be represented only in the discharge pattern of a subset of more sensitive fibers. Coarse gratings moving slowly across the skin (temporal frequency of 20 - 40 Hz) were best represented in the responding SA fiber population; fine gratings moving at a high velocity across the skin (temporal frequency > 100 Hz) were best represented in the discharge of the Pacinian fiber population, and stimulus combinations generating a temporal frequency in the range 30 - 100 Hz were precisely represented by discharge in the QA fiber population.

Specification of the mechanoreceptive fiber responses to the two-dimensional object pattern was found in a small data collection. With each successive stimulus presentation the moving surface was translated 125 microns laterally relative to the direction of movement. The response array generated by this procedure defined the representation of the stimulus surface both along an axis at right angles to its line of movement, and in the line of movement.
2385 OCULAR COUNTERROLLING DURING CONSTANT VELOCITY ROLL IN NORMAL AND PATIENTS WITH UNILATERAL VIII NERVE SECTIONS.
Shirley C. Diamond and Charles H. Markham. Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Nine subjects were rolled about their naso-occipital axes at a constant velocity of 3°/sec after having been securely strapped into the chair in various positions and stabilized by a bite bar. Trials began with a roll to 90° right ear down, reversed and rolled to 90° left ear down, again rolled to 90° right ear down, then back to 90° left ear down, then finally back to the upright baseline. At each 90° position, subjects were held steady for 30 sec before being rolled to the opposite direction. During the stationary intervals when subjects were held motionless at 90°, the eyes were photographed both eyes at each 10° of roll, and at each 10 sec while subjects were held motionless at 90° tilts.

Counterrolling was recorded by a dual projector system described in an earlier study (Diamond, Markham, Simpson & Curthoys, Acta Oto-Laryngologica, in press, 1979). Mechanical accuracy of the measuring device is 1 minute of arc; practical accuracy is 15 minutes.

Results in six normal subjects showed that contrary to general belief, the counterrotated position of the eyes is not held constant as long as the tilt is maintained. During the 30 sec intervals when subjects were held motionless at 90°, 28 observers were recorded. Of these, 22 of these showed the subjects' eyes continued to make occasional rotational movements. This torsion during the stationary interval was as much as 5°, with 2° being common. Direction of change during the stationary interval was not equally divided between more and less counterrolling. When subjects began rotation back toward the upright baseline, frequency torsion or "wrong" direction was greater. The amplitude of counterrolling was approximately equal on both sides.

Six patients with right vestibular nerve sections performed 2 to 5 years earlier showed distinctly abnormal patterns when they were rolled contralateral to the lesion, i.e. left ear down. These abnormal patterns consisted of reduced amplitude and/or more instability in the form of irregular "wrong" direction torsion, persisting longer than in the normal subjects and appearing closer to the upright position. Counterrotating was not conspicuously different from normals when patients were rolled to the side ipsilateral to the lesion. Further studies are underway to delineate the differences between normals and persons with unilateral lesions in aids to aid in the diagnosis of oticular dysfunction. (Partly supported by NASA NGR 05-007-418.)


In order to further analyze the somatotopic projection to SMI, single units were recorded from awake paralyzed, adult cats, using MgO analgesia and mechanical stimulation. Chronic recording chambers were implanted over the left SMI. Cells were classi­fied as to whether they responded to superficial (S cells), such as hair movement or light touch, or to deep stimulation (D cells), such as pressure or joint manipulation.

Of the 144 units studied during 9 control recording sessions re­vealed that SMI is organized in a specifically manner, with 93% of the cells belonging to a single submodality and having a definite receptive field. The surface is represented in SMI as a series of overlapping strips, oriented at about 45° relative to the craniocaudal sulcus, with rostral body segments lying caudal-laterally and caudal segments rostro-medially. D cells were concentrated rostro-laterally and S cells caudal-medially. Within each segmental strip, cells with dorsal RFs tended to be located caudal-medially while cells with ventral RFs were found rostro-laterally.

All dorsal roots caudal to L3, with the exception of L7, were sectioned for deafferentation. Adult cats, using MgO analgesia and mechanical stimulation. Chronic recording chambers were implanted over the left SMI. Cells were classi­fied as to whether they responded to superficial (S cells), such as hair movement or light touch, or to deep stimulation (D cells), such as pressure or joint manipulation.

The 144 units recorded during 12 control recording sessions re­vealed that SMI is organized in a specific manner, with 93% of the cells belonging to a single submodality and having a definite receptive field. The surface is represented in SMI as a series of overlapping strips, oriented at about 45° relative to the craniocaudal sulcus, with rostral body segments lying caudal-laterally and caudal segments rostro-medially. D cells were concentrated rostro-laterally and S cells caudal-medially. Within each segmental strip, cells with dorsal RFs tended to be located caudal-medially while cells with ventral RFs were found rostro-laterally.

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PATTERNS OF PROJECTIONS OF THALAMIC VENTROBASAL NEURONS TO THE FIRST SOMATIC SENSORY AREA OF MACAQUES. D.P. Friedman and E.G. Jones. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Cortical projections from physiologically identified neuropeptide within the thalamic ventrobasal complex (VPLc and VPM) were studied using the autoradiographic method. In each experiment the area of the thalamic receiving somatic sensory input was mapped using standard microelectrode recording techniques. A 1-µl Hamilton syringe was guided into VB according to these maps. The final position of the injection was selected by recording from a microelectrode which had been cemented to the barrel of the syringe so that the tip of the electrode was less than 500 µ from the bevel of the syringe barrel. This pair was placed into the barrel that was immediately expelled after determining the response properties of the nearby neurons. Such injections commonly produced a heavily labelled central core of less than 1 mm3. Virtually all of this zone was posterior and lateral to the syringe barrel, thus enveloping the recording site.

Injected clusters responding to stimulation of deeper tissues were almost always dorsally placed within VB, though some lateral projections were also observed. Neuron clusters were identified by the autoradiographic labelling as having been injected with leucine and proline (50 µci/µl, New England Nuclear) was expelled after determining the response properties of the nearby neurons. Such injections commonly produced a heavily labelled central core of less than 1 mm3. Virtually all of this zone was posterior and lateral to the syringe barrel, thus enveloping the recording site.

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The pattern of terminal labelling on the cortex was studied using series of 20 µ thin-sections contrasted autoradiographic sections cut in either sagittal or horizontal planes. Following injections into clusters responding to tactile stimulation labelling was confined to area 3b. Such labelling was seen in vertical columns or bands within layers IIIb and IV. An individual column could be produced by a single injection of marker from an adjoining column by an equivalent sized gap. Within 3b columns located on adjacent sections were often arranged in register so as to form a pattern of repeating strips or bands or variable orientation with respect to the axes of the postcentral gyrus. The more dorsal injection sites, placed where units responded to stimulation of deeper tissues, produced labelling mainly in areas 1 and 2. Within those areas labelled terminals appeared in elongated bands completely within layer III. Because the labelling in these areas was extremely light a finer structure could not be clearly resolved.

The nuclei of the trigeminal complex exhibit cytoarchitectonic and projectional differences which suggest the possibility of differential processing of somatosensory information. Physiological studies have provided contradictory results regarding the issue of trigeminal nuclear specialization. We are exploring this issue through a broad-based methodology using an extensive battery of precisely-controlled stimuli in conjunction with a variety of quantitative analytic procedures. Single-unit action potentials are recorded extracellularly with tungsten microelectrodes in the barbiturate-anesthetized albino rat. Stimuli consist of quantitatively controlled deflections of single mystacial vibrissae. Data are collected and analyzed by means of digital computers.

The responses of units of nuclei interopolaris and oralis are qualitatively similar. (1) About half the receptive fields include more than one vibrissa. (2) Angular displacement and velocity thresholds range over three orders of magnitude. (3) Adaptation rates vary widely and are highly dependent on stimulus amplitude.

Quantitative methods, however, reveal several differences. (1) Receptive fields of interopolaris units tend to be smaller than those of oralis. (2) Responses of interopolaris neurons are more robust than those of oralis neurons. About 1/3 as many interopolaris neurons as oralis neurons have receptive fields which include more than 10 vibrissae or a patch of skin to underlying tissues is maintained. Each mechanical stimulus to one spot is abolished when preceded at >50 µsec by another mechanical stimulus to the same spot. (3) Interopolaris neurons tend to adapt more rapidly than those of oralis neurons. The adaptation index (defined for a step deflection of about 7° for the receptive field) for interopolaris neurons is about 2/3 that of oralis neurons. Although stimulus-response relationships in nuclei interopolaris and oralis are qualitatively similar, quantitative differences suggest physiological specialization for differential processing of mechanical sensory information by these nuclei. (Supported by NIH Grant No. NS-14748.)
SOMATOSENSORY SYSTEMS

THE RELATIONSHIP BETWEEN THE PATTERN OF CONGUISM TERMINATIONS AND THE MAPS OF SOMATOSENSORY CORTEX (Sm I and Sm II) IN THE GREY SQUIRREL, H. J. Gould, III, Dept. Anat., Univ. Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Interhemispheric somesthetic connections were studied in the common ground squirrel using the HRP-labeling technique. The total pattern of connections was correlated with the electrophysiologcal maps of Sm I and Sm II. In six squirrels the corpus callosum was dissected into the medial surface of one hemisphere six days prior to mapping the contralateral somatosensory cortex. At the end of each recording session small reference lesions were made at known points within each map. The animals were then perfused and the tissue was flattened and sectioned parallel to the surface for analysis according to the technique of Walker and Woolsey (77, J. Comp. Neurol., 159: 137).

The densest degeneration is observed along the margins and in small areas within Sm I. This degeneration is related to the midline representations of the body including representations of the upper and lower lips, neck, trunk, genitalia and tail, while the representations of distal body parts include the vibrissae, corner of the mouth, forepaw and foot demonstrate little if any degeneration. Moderate degeneration is often seen related to midline border of the ventral horn. Neurons in this location, labeled by either the thalamic or the DCN injection, are large (40-65 µm) and are indistinguishable from the spinal border cells described by Cajal (1909), to collaterals ascending pathways to various supraspinal targets originate from the mesulam benzidin blue method revealed large (35-70 µm in diameter) multipolar cells scattered throughout the main body of the cortex. The contralateral projection is smaller, more numerous LTTD cells were not stained. This demonstrates that the innervation of the contralateral cortex provides a common function in the pyramidal and supraspinal systems. The organization of somatosensory neurons receiving trigeminal input arising from cutaneous mechanoreceptors has been the subject of many investigations. There have been few attempts to describe the organization of thalamic neurons responsive to cutaneous temperature stimulation of oral and perioral regions of the cat. H. Hirata, E.L. Auen, D.A. Pouls, and J.T. Moll, Dept. of Anatomy and Div. of Neurosurgery, Albany Med. Coll., Albany, N.Y. 12208.

The organization of thalamic neurons responsive to trigeminal tactile and cholinesterase by a two step cuprfate-ferricyanide method. In python, the large LTTD cells stained heavy for cholinesterase, while the small LTTD cells lack cells over 25 µm in diameter. However, the cells of the rattle snake RC showed heavy cholinesterase activity (25-45 µm in diameter). The large LTTD cells in python appear as a similar multipolar appearance as the large cells of the python LTTD. In both species, the large cells project to the contralateral tectum. Modification of the small cells exist in the python and may be a common function in the LTTD. A distinct nucleus in the rattle snake but not in the python. Supported by NIH grants ROI-EY01539, T32-EY00099, and by the Bell Telephone Laboratories.

SOMATOSENSORY SYSTEMS


The organization of thalamic neurons responsive to trigeminal input arising from cutaneous mechanoreceptors has been the subject of many investigations. There have been few attempts to describe the organization of thalamic neurons responsive to cutaneous temperature stimulation of oral and perioral regions of the cat. H. Hirata, E.L. Auen, D.A. Pouls, and J.T. Moll, Dept. of Anatomy and Div. of Neurosurgery, Albany Med. Coll., Albany, N.Y. 12208.

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Infrared receptors of pit viper and boid snakes project to a lateral mesencephalic nucleus of the lateral descending trigeminal tract (LTTD). Previously, using horseradish peroxidase (HRP) techniques, we showed that the LTTD of the python projects to the contralateral optic tectum via an intermediate nucleus in the ipsilateral ventro-lateral medulla, the nucleus reticularis calor is (RC). Now, using HRP, we have traced the LTTD projection to the tectum in the python (P. raticolatus). Following HRP injection into the intermediate layers of the tectum, the mesulam benzidin blue method revealed large (35-70 µm in diameter) multipolar cells scattered throughout the main body of the cortex. The contralateral projection is smaller, more numerous LTTD cells were not stained. This demonstrates that, in contrast to the case in rattlesnail, the python LTTD has a common function in both the pyramidal and supraspinal systems. The organization of somatosensory neurons receiving trigeminal input arising from cutaneous mechanoreceptors has been the subject of many investigations. There have been few attempts to describe the organization of thalamic neurons responsive to cutaneous temperature stimulation of oral and perioral regions of the cat. H. Hirata, E.L. Auen, D.A. Pouls, and J.T. Moll, Dept. of Anatomy and Div. of Neurosurgery, Albany Med. Coll., Albany, N.Y. 12208.

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ANATOMICAL DEMONSTRATION OF TRIGEMINAL AND SPINAL PROJECTIONS TO THE CAUDAL MEDULLA. Susan Rockfield and Stephen Gobel. Neurobiology & Anesthesiology Branch, HDHR, NBR, Bethesda, MD 20205.

The grey matter of the caudal medulla (CM) between the obex and the spinal cord is made up of dorsal and ventral horns which are lamellated much like the grey matter of the spinal cord. Earlier work has shown that layers I, V, and VI of the CM contain trigeminothalamic projection neurons that are likely to be involved in trigeminal pain pathways. Responses of layer I, V, and VI neurons to noxious stimuli can be modulated by electrical stimulation of several CNS sites. In the present study, neurons that project to the CM from the trigeminal nuclei are likely to be involved in trigeminal pain pathways. Responses of layer I, V, and VI neurons to noxious stimuli can be modulated by electrical stimulation of several CNS sites. In the present study, neurons that project to the CM from the trigeminal nucleus oralis are found in trigeminal nucleus oralis. The other trigeminal nuclei contain long distance projection neurons, i.e., layers I, V, and VI, suggesting that a single neuron may function as both a short intratrigeminal and a long distance projection neuron. Trigeminal nuclear oralis is the major source of descending intratrigeminal neurons to the CM which is an important criterion for distinguishing it from the main sensory nucleus and nucleus interpolaris. The propriospinal and intratrigeminal projections demonstrated in this study must constitute an important source of axonal endings in the CM which may modulate the activity of trigeminothalamic projection neurons.

INHILDIM DERMATOMES OF CAT USING AVERAGING METHODS. David H. Kohlinianska and Paul R. Brown. Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Previous reports of cat hindlimb dermatomes are so contradictory that they cannot be used with any precision. For proper mapping of dermatomes, the following criteria must be met: (1) Stimuli must consistently activate cutaneous nerves but not nerves innervating deep receptors; (2) The dorsal root recording method must be sensitive enough to detect activity in small number of fibers, activity in units in other dorsal roots; (3) A means of quantifying the response must be used; (4) Mapping of response magnitudes must use a consistent means of representation. Previous studies have generally failed to meet these criteria.

Seventy standard loci on the hindlimb were tested, in five adult cats, using mechanical pulses (3 msec rise and fall times) applied to the mat of clipped fur. An amplitude of 20 µm was used, since this was demonstrated in single unit studies to activate hair and cutaneous receptors. Averages were obtained from dorsal roots L4-S1, sampling at 10,000/sec (each root) and averaging over 100 trials (each root) at each stimulus point. Baseline dc offset, Ec, was computed from a pre-response period of 25 msec.

\[ E_{dc} = \frac{250}{250 - 1} \]

where \( I \) is the th sample point. The absolute-value noise level, \( E_{ao} \), was computed from the same sample points:

\[ E_{ao} = \frac{250}{250 - 1} \]

The response magnitude, \( E_{r} \), was computed from the next 250 sample points, which included the neural response.

Dermatomes were plotted as contour maps of response magnitudes on the hindleg, using three standard views for each dermatome. General locations agree with earlier reports, although individual reported dermatomes differ markedly from our results. There was little inter-animal variation.

This research was supported by grants from the West Virginia University Medical Center, and USPS Grant HS12061.


Precentral motor cortex responses to peripheral stimuli are attenuated when stimuli are delivered during large active movements (Evarts, E.V. and Fromm, C., Neurosci. Lett. 5: 267-272, 1977). One of the structures which might be involved in this attenuation is the post-central sensory cortex, a region reciprocally connected to motor cortex by cortico-cortical fibers. This hypothesis was examined by recording post-central neuron responses to stimuli delivered at various phases of active movement.

Monkeys (Macaca mulatta) grasped and supinated a handle in order to maintain accurate positioning of the handle in a target zone defined by a visual display. Movements were similar in magnitude (20°) and in velocity (150°/sec) to the "ballistic" movement which is not attenuated by post-central cortex responses. These results provide evidence for modulation of the responsiveness of post-central cortex neurons during active movement, and while not excluding modulation of peripheral input at subcortical levels, raise the possibility that post-central cortex is responsible, in part at least, for the modulation of peripheral inputs to motor cortex.

Supported in part by NIH International Research Fellowships P05-2597 (V.L.) and P05-2695 (R.V.)
CHANGES IN THE SENSITIVITY OF CUTANEOUS MECHANOCEPTION DURING SOMATOSENSORY SYSTEMS

mechanical stimulation. The threshold of the touch domes monitored on line with a photoelectric cell that was previously aged rats (over 6 months old) is significantly higher than the decreases with increasing age. The threshold of touch domes in receptors over the course of its life.

In addition to their density in the skin touch domes appear to alter their sensitivity to pressure as they age. The second mechanism involves an increase in threshold to mechanical stimulation as the animal reaches old age. The threshold of the touch dome increases with increasing age. The threshold of touch domes in aged rats (over 6 months old) is significantly higher than the threshold of touch domes in adult rats (5% days to six old). With decreasing density and increasing threshold, the rat progressively loses tactile sensitivity through these receptors over the course of its life.

LOCALIZATION OF LATERAL AFFERENTS IN THE CAT NODOSE AND CERVICAL SYMPATHETIC GANGLIA AS DETERMINED BY HRP AND FLUORESCENT DYE TRACER TECHNIQUES. G.E. Lucier and J.O. Dostrovsky. Faculty of Dentistry and Medicine, University of Toronto, Toronto, Canada M5G 1L6.

Previous electrophysiological studies (Mei, N. Exp. Brain Res. 11: 465, 1970; Sessle, B.J. Brain Res. 53: 333, 1973) have suggested that the nucleus of the solitary tract (NTS) cell bodies are mainly concentrated in the region of the entrance of the SLN to the nodose ganglion. In order to determine anatomically whether there is a specific localization of cell bodies of the nodose ganglion of the cat, axonal transport techniques employing fluorescent dyes and horseradish peroxidase (HRP) were used. In adult cats anesthetized with ketamine approximately 1 μl of 3% 4',6-Diamidino-2-phenyl indol-2HCl (DAPI) – 10% Prisuline in saline was injected into the right SLN, the nerve crushed at the injection site, and the animals allowed to recover. Four to five days later the animals were perfused with 10% buffered formalin and the nodose and superior cervical ganglia (SCG) removed. Frozen sections of the ganglia were cut at 30 μm and mounted on untreated slides. Sections were observed under a fluorescent microscope using a 360 nm excitation wavelength (van der Kooy, D. et al. Brain Res. 158: 189, 1978). Cell bodies containing DAPI-Prisuline could be easily distinguished from the dark background by their blue fluorescence. Labelled cells were maximally concentrated at the rostral end of the nodose ganglion and extended caudally along the border of the SLN entrance region. In the extreme rostral end single and small groups of labelled cells were seen scattered in the SCG and although more sparse and scattered tended to be localized unilaterally along the periphery of the ganglion. Injection of HRP into the laryngeal wall resulted in a similar distribution of HRP-labelled cell bodies in the nodose ganglion. These studies indicate that the cell bodies of SLN are localized within specific regions of the nodose and cervical sympathetic ganglia (Supported by NIH and Canadian MRC).


Mountcastle and Powell’s demonstration that a systematic relative relationship exists between sensory receptor structure and sensory central cortical organization has been confirmed repeatedly by other workers investigating the organization of the postcentral cortex in primates. The cytoarchitectural boundaries also exist within the parietal cortex occupying the superior bank of the Sylvian sulcus in macaque monkeys (Macaca mulatta and Macaca fascicularis) near the functional boundaries of the second somatosensory cortical projection field (S-II/r), and (ii) to obtain neurophysiological data that would provide information about the functional meaning of these transitions. In neurophysiological mapping experiments, we determined the receptive field locations and response properties of single neurons isolated during microelectrode penetrations that crossed the boundaries of interest. The information obtained was plotted along the electrode tracks identified in serial sections from each experimental brain. The boundaries between the hand representation and the neighboring representations of proximal body regions (arm and face) in S-II/r coincide with clearly visible cyto- and myeloarchitectonic boundaries. (In most olfactory or cortical sections through S-II/r, there are at least two such bands – one superior and one inferior of the hand representation.) To obtain additional support for the existence of transitions in cytoarchitectural and functional terms, we used the retrograde tracer horseradish peroxidase (HRP) to identify the axonal pathways of single-neuron recordings of the regions of the contralateral S-I and S-II/r; ipsilateral connections were identified by HRP injections performed in functionally identified regions of the ipsilateral S-I. The tissues were processed using the Hanks-Yates procedure. Analyses of the distribution of labelled cells revealed that (i) the neurons within the hand area of S-II/r do not extend to the opposite S-I or S-II/r, many neurons in this area send axons to the ipsilateral S-I hand representation; and (ii) the boundaries of the sciotic S-I/r area determined by retrograde labelling correspond precisely with the boundaries of the hand area as determined neurophysiologically. (Supported by NS10865, DE02668, RR05331, and NS1757.)

LOCALIZATION OF LATERAL AFFERENTS IN THE CAT NODOSE AND CERVICAL SYMPATHETIC GANGLIA AS DETERMINED BY HRP AND FLUORESCENT DYE TRACER TECHNIQUES. G.E. Lucier and J.O. Dostrovsky. Faculty of Dentistry and Medicine, University of Toronto, Toronto, Canada M5G 1L6.

Previous electrophysiological studies (Mei, N. Exp. Brain Res. 11: 465, 1970; Sessle, B.J. Brain Res. 53: 333, 1973) have suggested that the nucleus of the solitary tract (NTS) cell bodies are mainly concentrated in the region of the entrance of the SLN to the nodose ganglion. In order to determine anatomically whether there is a specific localization of cell bodies of the nodose ganglion of the cat, axonal transport techniques employing fluorescent dyes and horseradish peroxidase (HRP) were used. In adult cats anesthetized with ketamine approximately 1 μl of 3% 4',6-Diamidino-2-phenyl indol-2HCl (DAPI) – 10% Prisuline in saline was injected into the right SLN, the nerve crushed at the injection site, and the animals allowed to recover. Four to five days later the animals were perfused with 10% buffered formalin and the nodose and superior cervical ganglia (SCG) removed. Frozen sections of the ganglia were cut at 30 μm and mounted on untreated slides. Sections were observed under a fluorescent microscope using a 360 nm excitation wavelength (van der Kooy, D. et al. Brain Res. 158: 189, 1978). Cell bodies containing DAPI-Prisuline could be easily distinguished from the dark background by their blue fluorescence. Labelled cells were maximally concentrated at the rostral end of the nodose ganglion and extended caudally along the border of the SLN entrance region. In the extreme rostral end single and small groups of labelled cells were seen scattered in the SCG and although more sparse and scattered tended to be localized unilaterally along the periphery of the ganglion. Injection of HRP into the laryngeal wall resulted in a similar distribution of HRP-labelled cell bodies in the nodose ganglion. These studies indicate that the cell bodies of SLN are localized within specific regions of the nodose and cervical sympathetic ganglia (Supported by NIH and Canadian MRC).


Central connections of sensory trigeminal nerve branches were identified in the cat trigeminal brainstem complex (main sensory nucleus and spinal trigeminal nucleus) from the frontal, infraorbital, or mental nerve was cleanly transected and anchored in HRP-filled microtubule (SO, Bühner-Mahmen). Forty-eight to ninety-six hours postoperatively, the animals were perfused according to the methods of Roseme and Mesulam (1978). The tissues were reacted with tetramethylbenzidine (TMB), mounted on slides, and examined under darkfield microscopy for the presence of HRP reaction product. Retrograde transport of HRP in transected fibers of the frontal, infraorbital, or mental nerves, respectively, injected into parent cell bodies in the anteromedial, intermediate, and postcentral regions of the ipsilateral trigeminal ganglion. Transperforatorial transport of HRP was detected within the central processes of these primary trigeminal neurons and was used to trace their terminations in the trigeminal brainstem complex. These central processes terminated somatotopically in the main sensory and spinal nuclei, with the mental nerve fibers terminating most dorsally, frontal fibers most ventrally, and infraorbital fibers primarily in an area between the latter two. The infraorbital nerve fibers were present in greater numbers and projected in modest amounts to the dorsal and ventral areas of S-II/r. Labeled fibers from all three nerves terminated throughout the rostrocaudal extent of the trigeminal brainstem complex. However, fibers were observed in the main sensory nucleus and the rostral part of pars caudalis. Labeled processes or terminals could be found in the marginal, substantia gelatinosa, and magnocellular layers of pars caudalis but were most numerous in the magnocellular layer. Few labeled fibers could be identified below the first cervical segment. Individual fibers entering the trigeminal sensory nucleus were occasionally observed coursing within intranuclear fiber bundles. However, the majority of entering fibers were seen in relation to, and presumably terminating in, areas containing nerve cell bodies. No labeled fibers were seen passing to the contralateral trigeminal complex.

Central connections of other branches of the trigeminal nerve are currently under investigation in this laboratory in an effort to refine our knowledge of somatotopic organization in the trigeminal brainstem complex. (Supported by Biomedical Research Grant RR054)

The extracellular responses of single units in SmI of 4 awake, paralyzed, adult cats under N2O:O2 anesthesia were examined before, during, and after induction of an epidural anesthetic, represented here by the local anesthetic bupivacaine (Bupiv). Blocks of the L4-L5 dorsal roots. Chronic recording chambers were implanted over the left SmI and units were isolated in the lateral-medial region of SmI. Unit responses were recorded to different projections from the contralateral upper hind limb. The somatotopic representation and response properties of the 213 units sampled were similar whether the animal was paralyzed or not, but the layer of origin of the corticothalamic projection was different in each animal. The corticothalamic projection was observed in all 4 cats and in general agreement with the results of other studies. Nine units were recorded from the contralateral hind limb. Some of these units were located in the lateral-medial region of SmI and others were located in the secondary representation of the hind limb. The layer of termination of the thalamocortical projection is distinct for different thalamic nuclei.


We demonstrated previously in the cat that afferents from the three trigeminal divisions and from the C2 and C3 dermatomes project rostrally to the marginal zone of the spinal cord. These afferents terminate in the gray matter of the medullary-spinal cord transition zone (Nord, S.G. et al., Neurosci. Abstr., 1978, 4: 556). The present experiments were undertaken to extend these observations to other regions of the marginal zone extending from 5.1-8.5 mm caudal to the obex was explored in the monkey for neurons which responded to mechanical stimulation of the face and body. In general, microscopic observations were made at, or just rostral to, levels at which the C2 dorsal roots could be seen entering the spinal cord. The locations of labeled neurons were determined histologically in laminae IV-VI. Although some responded only to noxious stimulation and others to light touch, more (63%) were of the "wide dynamic range" type, responding differentially to variations in stimulus intensity. The majority of the neurons (62%) had receptive fields which lay entirely within the facial representation, however, such units decreased with distance from the obex. These fields were innervated predominantly (89%) by afferents from the ophthalmic dermatome, and all but one were restricted to the intermediate and peripheral zones of the classical main-skin facial pattern. An additional 29% of the neurons had fields which extended to adjacent dermatomes. (Supported by NIH grant 10814.)


The neocortex of Galago can be divided into three sensory fields, visual, auditory and somatic (Diamond, 1979), each of which is composed of several subdivisions. The somatic field consists of Brodmann's area 4, a premotor area, a somatocortical area, and a belt area between area 4 and the somatocortical area. We studied thalamicocortical connections of single subdivisions of the somatic field by making small intracellular injections of horseradish peroxidase (HRP) or a mixture of horseradish peroxidase and horseradish peroxidase is reduced to a diffuse reaction product with H2O2 and form. The principal finding is that the labeled cells or terminals in the thalamus are distributed in bands which cross nuclear borders and many other CNS structures. This may be related to the fact that the neurons mediating afferent thalamicocortical projections are distributed in bands which cross nuclear borders and/or other CNS structures.
2407 EFFECTS OF MECHANICAL STIMULUS FORCE ON DISCHARGE OF RACCOON GLABROUS SKIN SLOWLY ADAPTING CUTANEOUS MECHANOCEPTORS. Benjamin N. Pubols Jr., Christine M. Malinak* and Ann F. Corson*. Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, Penna.

When a mechanical stimulus produces a constant displacement of raccoon glabrous skin, concomitant reactive force reaches its peak just as the controlled static displacement is achieved, and then declines slowly. The reactive force is steady for a study level of 20 g. In contrast, when a constant force is applied, skin displacement may continue to increase over a period of several minutes. Upon suction, the comparable duration elicited is 3 to 6 minutes. It is required for the skin to return to its original resting position. Skin displacement is adequately described as a power function of applied force. The slopes of the functions change for the force range 1-20 g, both initially and 20 sec after static force onset. Within this force range, displacements of 50 to more than 2000 µ are generated.

To study the role of force in determining slowly adapting (SA) mechanoreceptive afferent fiber discharge, individual median nerve fibers were isolated by microdissection in 10 sodium pentobarbital anesthetized raccoons.

The median absolute force threshold was 560 mg (range = 70-120 mg), while the median absolute displacement threshold was 20 µ (range = 5-185 µ). Absolute force and displacement thresholds were positively correlated (N = 27; P < .01).

For most moderately slowly adapting (MSA) and very slowly adapting (VSA) units a power function (exponents between 0.62 and 1.23) provided the best fit (highest r), while for a majority of VSA units a logarithmic, and power functions were fit to the stimulus-response data of 10 MSA and 6 VSA units. For a majority of MSA units a power function provided the best fit (r^2 = 0.62 and 1.23) provided the best fit (highest r), while for a majority of VSA units, a logarithmic function provided the best fit.

Results of the present investigation indicate that static discharge of glabrous skin slowly adapting cutaneous mechanoreceptors is in part dependent upon the viscoelastic properties of skin, and suggest that force may be more potent than displacement in determining the behavior of such receptors. (Supported in part by research grant NS-13418, USPHS).

2408 SYMPATHETIC MODULATION OF SENSITIVITY IN CAT CUTANEOUS MECHANO-RECEPTORS. William J. Roberts and Joseph P. Pierce*. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97209.

Sympathetic efferent control of afferent activity has been studied in two different cutaneous mechanoreceptors in anesthetized cats: "type II" receptors and 6 hair receptors. Single units were functionally identified and then stimulated mechanically with a solenoid-driven probe to establish a baseline response. During this mechanical stimulation the sympathetic trunk was stimulated electrically and any sympathetic influence on firing was noted. Changes in activity unrelated to a constant stimulus amplitude or as a change in stimulus intensity required to maintain a constant level of activity.

Type II receptors most commonly showed a marked increase in tonic discharge but no change in sensitivity to mechanical stimuli. This increased activity was often maintained throughout 3 min of sympathetic stimulation, although some variation was seen between units. Sympathetically-induced changes in the sensitivity of 6 hair receptors were related to the velocity sensitivity of individual units. The most rapidly-adapting (6g) were generally desensitized, while the least rapidly-adapting (6g) were sensitized. Intermediate units (6g) were least affected. The measured changes in sensitivity of affected units were commonly on the order of 10-30% of the unconditioned threshold, although some were much larger. Sympathetic activity thus appears to result in a shift in the sensitivity of 6 hair receptors, enhancing the detection of slower hair deflections relative to rapid deflections. As with most of the "special" senses previously tested, cutaneous sensibility in mammals is now demonstrated to be subject to efferent control.

2409 DINECEPHALIC PROJECTIONS OF THE PONTINE RETICULAR FORMATION. Richard T. Robertson. Department of Anatomy, College of Medicine, University of California, Irvine, CA, 92717.

Ascending projections from the pontine reticular formation were studied in the cat with autoradiographic techniques. Small injections of 3H-leucine were placed in either the rostral (n. reticularis pontis oralis) or caudal (n. reticularis pontis caudalis) divisions of the large caudal pontine reticular formation. Pathways of ascending fibers and patterns of termination were studied in sections cut in the transverse or sagittal planes.

Efferent projections from both rostral and caudal pontine regions ascend to the caudal diencephalon and appear to bifurcate into two branches, as has been described previously. A dorsal branch terminates in the intralaminar nuclei of the dorsal thalamus, and a ventral branch terminates in the subthalamic region. Termination sites in the dorsal thalamus include the centre median, central lateral, paracentral, and subthalamic region, but the relative densities of these two general regions of termination are generally desensitized, while the least rapidly-adapting (6g) were sensitized. Intermediate units (6g) were least affected. The measured changes in sensitivity of affected units were commonly on the order of 10-30% of the unconditioned threshold, although some were much larger. Sympathetic activity thus appears to result in a shift in the sensitivity of 6 hair receptors, enhancing the detection of slower hair deflections relative to rapid deflections. As with most of the "special" senses previously tested, cutaneous sensibility in mammals is now demonstrated to be subject to efferent control.

2410 ULTRASTRUCTURAL CHARACTERIZATION OF NORADRENERGIC AXONAL ENDINGS IN LAYERS I AND II OF THE DORSAL HORN OF THE MEDULLA. M.A. Ruda, N. Allen* and S. Gobel. NAB, NIDR, NIH, Bethesda, MD 20205.

Recent studies have drawn attention to the role of monoamines in the modulation of the response of nociceptive neurons in the dorsal horn. Previous experiments in our lab have characterized serotonergic (5HT) axonal endings in layers I and II of the dorsal horn (DHM). The purpose of the present experiment was to similarly identify noradrenergic endings. Our approach accesses the ability of neurons which use NE as a neurotransmitter, to take up triitated norepinephrine ([3H]NE) at their axonal endings. Adult cats, pretreated with a monoamine oxidase inhibitor, were anesthetized with sodium pentobarbital and [3H]NE (10-5M) topically applied onto the DHM for 1 hr. In one experiment, 2 hrs prior to the [3H]NE application, a serotonin neurotoxin (10^4 M 5,6 dihydroxytryptamine (5,6 DHT)) was topically applied for 1 hr. 5,6 DHT causes degeneration of 5HT axonal endings. Thirty min after application of the [3H]NE, the cats were perfused and the DHM processed for autoradiography. Analysis of light microscopic autoradiographs demonstrated an accumulation of [3H] NE in layers I and II. In EM autoradiographs, two categories of labeled axonal endings were identified: dome-shaped endings which form synapses on a single postsynaptic structure and scalloped endings which form synapses on several postsynaptic structures. These categories were further divided into five types based on morphological criteria. The same five types of labeled endings were observed in the experiment combining 5,6 DHT and [3H]NE and in the experiment in which only [3H]NE was used as a marker. Three types of dome-shaped endings were observed and in both experiments the presence of either plasmalemmal, small oval or large oval agranular vesicles. They formed mainly asymmetrical synapses on dendritic spines and shafts. In several cases, the same dendrite, received synapses from both a degenerating 5HT ending and an [3H]NE Labeled ending. One type of scalloped ending was observed in both in layers I and II and contained large oval agranular vesicles while a second type was found only in the deeper part of layer II. Both types of endings form synapses on dendritic shafts and spines. The presence of [3H]NE labeled axonal endings in layers I and II of the DHM suggests that NE affects have act upon both the ascending noradrenergic neurons in layer I and the interneurons in layer II. The observation of both SHT and NE endings synapping on the same dendrite further suggests that both monoaminergic systems modulate the output of layer I projection neurons through similar mechanisms.

Collateral branching of cortical neurons projecting to the dorsal column nuclei has been investigated with a double-labeling technique, using RHP and 3H-apo-RRP as distinguishable retrograde tracers (Hayes and Rustioni, 1978). In cats and rats, the tracer was injected into the brachial lateral aspect of the spinal cord, and the other tracer was injected into the ipsilateral dorsal medulla. In rats, cervical spinal injections were made through the sensorimotor cortex, while in the cat the injection was into the thalamus. All animals were sacrificed by perfusion with mixed aldehydes after 48 hours. One mm thick slabs through the sensorimotor cortex were processed with Hanker-Yates embedding, embedded in parafit, sectioned at 15µm, and processed for autoradiographic demonstration of 3H-apo-RRP.

In the rat, corticospinal neurons were concentrated in layer V in a band which extended approximately 7 µm along the dorsomesial aspect of the contralateral hemisphere (areas 3, 4, 6 and 7 of Krieg). Neurons labeled by injections in the dorsal column nuclei were found throughout layer V along a similar rostro-caudal extent and were most numerous in more lateral parts of the cortex. While the locations of the cells of origin of these two descending pathways were, for the most part, segregated (in part due to the large face representation in the lateral hemisphere of the rodent), many neurons double-labeled both corticospinal and thalamic fibers descending to lower levels in the dorsal part of the lateral funiculus. Therefore, in one cat the spinal injection was made into the lateral medulla and retrograde labeling of corticospinal neurons projecting to both cortical and lumbar levels, while in the cat the thalamic injection was targeted to the thalamus. All animals were sacrificed by perfusion with mixed aldehydes after 48 hours. One mm thick slabs through the sensorimotor cortex were processed with Hanker-Yates embedding, embedded in paraffin, sectioned at 15 µm, and processed for autoradiographic demonstration of 3H-apo-RRP.

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PATTERNS OF REINNervation following replantation of the RABBIT EAR. R. G. Turnbull and J. E. Teras. Microsurgical Research Laboratory, McGill University, Montreal, Quebec.

Refinements in microsurgical techniques over the past decade have improved the surgical management of peripheral nerve lesions and have permitted replantation of free composite tissues. However, cutaneous reinervation following replantation or nerve repair often remains abnormal. The aim of this work was to employ cutaneous techniques and study the changes of the reinnervated mechanoreceptors in a normal animal model. The results demonstrate that the size, shape and areas of innervation were similar to those of the normal animal.

The right ears of 30 New Zealand white rabbits were replanted and studied at various post-operative periods ranging from two weeks to eight months. The ear was re-exposed proximal to the nerve repair. Single fibres were isolated by dissection and their signals were studied using standard electrophysiological equipment. Once a single active area was isolated, the ear was searched for its receptive field (RF). The size, shape and relative proportions of the several types of RF were studied as a function of innervation age.

The number of active areas within each RF was recorded as were details of its response characteristics. Finally, the conduction latencies of the fibres were obtained by percutaneous electrical stimulation.

Reinnervated RF's differed from normal fields by (i) being smaller and more irregular in contour, (ii) having fewer hairs innervated by a single nerve fibre, (iii) exhibiting changes in the relative population of hair units versus skin units and by displaying multiple skin areas served by one fibre. Although both the size of the RF's and the number of hairs capable of activating the fibre increased with time, reinervated RF's seldom achieved normal dimensions, and irregularities in shape and hair field were often pronounced. The size of this work was to explore the relationship between the size of the RF and the number of hairs innervated by the fibre. Higher values were observed than in normal ears; however, the correlation between the two was not significant. The number of hairs innervated by the fibre was found to be significantly lower in reinervated fields than in normal ears. The results demonstrate that the size, shape and areas of innervation were similar to those of the normal animal.
Two *Macaca nemestrina* monkeys have been trained to identify the location of a tactile stimulus delivered to the glabrous skin of either foot. The monkeys initiate trials by pressing a lever and holding until a pulsatile tactile stimulus (10 Hz) begins. Release of the lever within 1.5 sec of stimulus onset starts a response period in which a press of one of 6 buttons on a panel produces liquid reinforcement. On the panel surface facing the animal is an artist's rendering of the glabrous surfaces of a monkey's feet. One of several panels is selected for a daily session; the panels differ in the location of the six response buttons. Panel A, presented during preoperative training and early postoperative testing, has 3 buttons on each foot, located over the distal phalanx of the 4th toes, the middle of the soles and the heels. During blocks of 100 trials within sessions, three stimulus probes (1.5 mm diameter) are positioned over different combinations of locations on the feet that are represented by buttons on the panel, and the correct button response on each trial is defined by the location of the stimulator driven. For the combinations used with panel A, 2 monkeys have performed preoperatively at better than 95% correct button responses, and a left dorsal column (DC) lesion in one animal has deteriorated performance slightly at only one of the points (85% correct responses to stimuli of the ipsilateral mid-sole location). In addition, bar release latencies and button response latencies are unchanged by the lesion. The lesioned monkey has been trained postoperatively, using panel B, to respond accurately to stimulation of the distal pads of toes 2 or 4 or the base of toe 4 on either foot. There is no detectable ipsilateral deficit in performance on this panel. Thus, despite severe deafferentation by buttons on the panel, and the correct button response on each trial is defined by the location of the stimulator driven. For the combinations used with panel A, 2 monkeys have performed preoperatively at better than 95% correct button responses, and a left dorsal column (DC) lesion in one animal has deteriorated performance slightly at only one of the points (85% correct responses to stimuli of the ipsilateral mid-sole location). In addition, bar release latencies and button response latencies are unchanged by the lesion. The lesioned monkey has been trained postoperatively, using panel B, to respond accurately to stimulation of the distal pads of toes 2 or 4 or the base of toe 4 on either foot. There is no detectable ipsilateral deficit in performance on this panel. Thus, despite severe deafferentation of the distal extremity portion of SI cortex in monkeys by DC lesions, single stimuli to the feet can be quite accurately localized following DC section. Supported by grants NS 11132 and NS 14899.

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SPINAL CORD

The efficacy of steroids in protecting the spinal cord (SC) from blunt trauma is problematic. Disparity in results may exist due to differences in (a) experimental animals (b) severity of injury (c) doses of steroids administered (d) techniques for evaluating neurological recovery, or (e) length of recovery period. The purpose of the present study was to determine the effects of a high dose of methylprednisolone sodium succinate in ameliorating the effects of SC compression trauma. For this study immobilized & conditioned female mongrel cats ranging in weight from 2.5 to 4.0 kg were anesthetized with pentobarbital sodium (30mg/kg ip). All cats were immobilized in a stereotoxic frame, a one-segment laminectomy performed & L2 injured & post-operatively treated as described previously. (J. Neurosurg. 44:715-722, 1976). The SC's of 23 cats were compressed with 170gms for 5 minutes. Starting 2 hrs post injury, 16 of these cats were given methylprednisolone (30mg/kg body wt) for 3 days, 150mg/kg body wt for the next 3 days & 7.5mg/kg body wt. for the final 3 days of a total 9 day regimen. The remaining 7 cats served as injured but untreated controls. Neurological function was evaluated in all cats bi-monthly for a 2 month recovery period after which they were sacrificed by intracardiac perfusion of 10% formalin & the SC's removed for histologic examination. Our neurologic evaluation procedure is based on a 16 point scale with 16 being normal. Methylprednisolone treated cats showed significantly (p<0.001) earlier recovery (total score: 5.9 ± 0.3 vs 11.4 ± 0.3 at 2 weeks and 1 mo. respectively vs 3.7 ± 0.4 & 8.0 ± 1.0 at the same time intervals for controls) & complete recovery (total score: 14.1 ± 0.3 at 2 mo. vs 11.1 ± 0.5 at 2 mo. for controls) than control animals. Histologically, SC's from steroid treated animals consistently showed greater tissue preservation than those from controls. Our data clearly shows that long term high dose methylprednisolone treatment is effective in protecting feline SC tissue from post injury auto-destruction. However, its potency in this regard may depend on the extent to which methylprednisolone reaches the injury site. We suggest that this is a function of the level of post injury SC blood flow which has been shown to be inversely related to the magnitude of compression (J. Neurosurg. 45:660-667, 1976). Supported in part by the VA and grants from the Paralyzed Veterans of America & the Upjohn Co.

2422 DISTRIBUTION OF MET-ENKEPHALIN, SUBSTANCE-P AND SOMATOSTATIN IMMUNOREACTIVITY IN THE SPINAL CORD DORSAL HORN AFTER HEMISECTION IN THE RAT. M.S. Beattie, J.C. Bresnahan, R. Ho and F. Liuzzi*, Dept. Anat., Sch. Med., The Ohio State University, Columbus, OH. 43210.

Met-enkephalin (ENK), substance-P (SP) and somatostatin (SOM) -like immunoreactivities have been described in the dorsal horn of a number of species, and a role for these peptides in the transmission and modulation of nociceptive information has been postulated. In a preliminary attempt to assess the effects of chronic disruption of the ascending components of the pain modulating system, we have evaluated the effects of thoracic hemisection of the spinal cord dorsum. Bilaterally in a transverse, 2 dimensional grid of recording loci 0.2 mm apart mediolaterally and 100 µ or 200 µ apart dorsally in adult cats maintained on methoxyfluorane anesthesia, using platinum iridium microelectrodes. The dorsal horn was mapped for penetrations containing loci responsive to nerve stimulation overlapped with that described above but was slightly larger. The indirect immunofluorescent and modifications of the indirect PAP techniques were employed to demonstrate ENK, SP and SOM immunoreactivities on semi-adjacent sections in animals allowed to survive 1, 3, and 5 days, and 3, 5 or 20 weeks following spinal hemisection. Cross reactivity tests and absorption controls demonstrated specificity of immunostaining for each of the antisera used.

The pattern of distribution in the superficial laminae of the dorsal horn was comparable to that reported by other authors from each of the three peptides. ENK, SP, and SOM immunoreactivites were observed in varicosities and terminals within both laminae I and II. Overlap between the distributions was substantial with ENK exhibiting the deepest penetration into the dorsal horn. Although consistent asymmetries were observed between the two sides of the spinal cord, these differences were not consistently related to the surgical treatment. These results suggest first, that the majority of ENK, SP, and SOM immunoreactivities in laminae I and II is related to segmental systems in the spinal cord (i.e. dorsal roots and interneurons). Secondly, the results suggest that in the rat, unilateral disruption of descending systems does not produce a reorganization of these peptidergic systems of sufficient magnitude to produce obvious changes in their distribution as revealed by these immunohistochemical techniques. A report by Naftchi et al. (Brain Res., 153: 121-128, 1978) also observed fairly dramatic changes in SP distribution following spinal section in the cat, may be indicative of a species difference in this regard. Studies are currently underway to examine this possibility. (Supported by N.I.H. Grants NS-14457 and NS-10165.)

2423 SURAL NERVE INPUT TO THE DORSAL HORN IN NORMAL AND ACUTOY HEMISECTED CATS. Gene L. Brownowitz* and Lillian M. Pobuls, Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Axonal sprouting of dorsal root afferents is known to occur following partial hemisection of the spinal cord (Murray and Goldberger, J. Comp. Neur. 156:19-36, 1974). Our long term goal is to examine changes in cutaneous input to the dorsal horn correlated with this type of sprouting.

This report describes the distribution of dorsal horn neurons responding to electrical stimulation of the sural nerve in normal and acutely hemisected cats. The responses of single units and unit clusters in the L7 segment to electrical stimulation of the sural nerves and to natural tactile stimulation were studied in adult cats maintained on methoxyfluorane anesthesia, using platinum triduum microelectrodes. The dorsal horn was mapped bilaterally in a transverse, 2 dimensional grid of recording loci 0.2 mm apart mediolaterally and 100 µ or 200 µ apart dorsally. Some cats received right (low thoracic or high lumbar) hemisections, sparing the dorsal columns, 2 or 3 days prior to recording.

Sites yielding reliable short latency (< 5msec) responses to electrical pulses were clustered in the lateral one half of the dorsal horn, largely in laminae IV-VI. Natural receptive fields for penetrations containing loci responsive to nerve stimulation always included some part of the region extending from the heel to the ventral foot just above the base of digit 4. The region of the dorsal horn yielding longer latency (> 5msec) responses to sural nerve stimulation overlapped with that described above but was slightly larger.

In acutely hemisected cats it was more difficult to delineate natural receptive field boundaries on the lesioned side due to an increase in spontaneous activity in the laminae responding to tactile stimuli. Our preliminary findings indicate, however, that there are no striking changes in somatotopy or in the location and extent of the region responsive to sural nerve stimulation as a result of hemisection. Supported by: NIH Grants NS 13677 and NS 07061.
2425 ULTRASTRUCTURE OF SYNAPTIC TERMINALS IN LAMINAE I AND II OF THE CAT SPINAL CORD AFTER ANTEROGRADE LABELLING OF DORSAL LATERAL FUNICULUS AXONS WITH HRP. J.C. Bresnahan and M.S. Beattie, Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

Axons descending from the brainstem raphe nuclei and adjacent reticular formation probably participate in the modulation of neuronal activity in the marginal layer and substantia gelatosa of the spinal cord (laminae I and II). These fibers have further been shown to travel in the dorsal lateral funiculus (DLF) of the cord in cat, rat, monkey, and opossum. We have labelled axons in the DLF using anterograde injury-filling with HRP and examined the synaptic terminals of these fibers in laminae I and II.

Adult cats were anesthetized with sodium pentobarbital and maintained as described previously (Beattie et al., Brain Res., 153: 127-134, 1978). Microinjections of HRP were made in the DLF either hydratologically or iontophoretically; the damage caused by the injection pipette was sufficient to produce considerable injury filling of axons which were confined to the DLF with little, or no contamination of adjacent structures. Alternatively, a small cut in the DLF was made and dried pellets of HRP were introduced into the cut. Tissue was processed using DAB as the reaction substrate with cobalt chloride intensification. Survival time was from 18 to 24 hours.

With the light microscope, labelled axons within the DLF could be traced back to a cutaneous injection site. Many entered laminae IV-VII in a dense projection presumably representing cortico-spinal, rubro-spinal, and other descending systems. A smaller number of relatively fine-caliber axons could be traced to laminae I and II with many exhibiting a transverse orientation capping the dorsal horn. Terminal and en passant swellings were easily visible. Although both small myelinated and unmyelinated axons were labelled with HRP in laminae I and II. The majority of synaptic terminals labelled with HRP contained round vesicles and a few dense core swellings were easily visible. With the electron microscope, similar morphology were degenerating caudal to the region of HRP labelling. Both the HRP-labelled and the degenerating terminals were comparable to the types of degenerating terminals observed by Goode et al. (1977) after lesions of raphe magnus in the opussum. The relationship of these terminals to those of a primary afferent origin (Beattie et al., 1978) will be discussed. (Supported by N.I.H. Grants NS-14457 and NS-10165.)


HRP as a retrograde marker has done much to revolutionize the way we visualize neuronal connectivity in the central nervous system. Although extremely useful, the technique is time consuming and not easy to use in double labelling experiments (i.e., titrated but inactivated HRP-regular HRP). Recently several authors have reported success using fluorescent dyes as retrograde markers and because of the potential use of dyes for double labelling we have tested their effectiveness on systems of interest to us. The fluorescent dye 4,6-diamino-2-phenylindole (DAPI) appears to be particularly good. Following injection of DAPI into the cord we have seen evidence for brainstem labelling which appears comparable to that present to other animals processed via the HRP method. The animals with dye injections were sacrificed two to four days after surgery and the unfixed brains were removed for cryostat sectioning. The sections were examined with a fluorescence microscope and labelled cells could be seen using a 360nm. filter system. Such cells could be traced back to a pool of oil where the sciatic nerve (for identification of lumbar spinal cord) had been lesioned bilaterally days or more previously, CBZ, even at high doses (10 mg/kg i.v.) had only minimal effects on muscular rigidity. It is suggested that the clinical beneficial effects of CBZ could be mediated by an interaction with the descending coeruleospinal noradrenergic inhibitory projection.


The locus coeruleus (LC) provides a massive noradrenergic projection to the ventral horn (VH) of the spinal cord (Commissiong et al., 1978). Cyclohexenprine (CBZ), a centrally-acting muscle relaxant released a dose-dependent depletion of norepinephrine (NE) in the VH of the lumbar spinal cord. However, noradrenergic transmission in the cord, as assessed by the production of methoxyphenylglycol (MPG), the major metabolite of NE, remained intact, even when NE was depleted by greater than 80%. All analyses were done by a gas chromatographic-mass fragmentographic method. CBZ (1.5 mg/kg i.v.) completely abolished muscular rigidity of the gastrocnemius-soleus muscle group in the intercollicular decerebrate rat. Muscular rigidity was assessed by an electromyographic method. However, in rats in which the TD had been lesioned bilaterally 8 days or more previously, CBZ, even at high doses (10 mg/kg i.v.) had only minimal effects on muscular rigidity. It is suggested that the clinical beneficial effects of CBZ could be mediated by an interaction with the descending coeruleospinal noradrenergic inhibitory projection.


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2. Cyclohexenprine was generously supplied by Merck Sharp and Dohme.
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EFFECTS OF L-DOPA ON DORSAL HORN UNIT RESPONSES TO NOXIOUS THERMAL STIMULATION. Jonathan Delatizky, Charles J. Hodge and Charles I. Woods*. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, N.Y. 13210. 

Descending noradrenergic pathways are believed to participate in modulation of spinal nociception. Stimulation of locus coeruleus has been reported to cause inhibition of spinal nociceptive cells, although interactions with known inhibitory serotonergic pathways may contribute to this effect. Direct iontophoretic application of noradrenaline (NA) or serotonin (5-HT) on spinal nociceptive cells is inhibitory. In contrast, nociceptive thresholds have been shown to increase following NA depletion, suggesting that NA may enhance nociception. We previously described a facilitatory role for NA on cells responding exclusively to innocuous stimuli. The contribution of NA to spinal sensory modulation is consequently uncertain. 

We have investigated the effects of L-DOPA induced terminal NA overflow on responses of lumbar dorsal horn units to noxious thermal stimuli. NA actions have been separated from those of other transmitters and metabolites. Unit responses to noxious thermal stimuli were recorded extracellularly in acute spinal cats. A Peltier-effect thermode with feedback control was used to vary the skin temperature between 25 and 36°C in a standard stepped sequence. Responses to this sequence were recorded every 3 to 10 minutes at least twice, and for at least an hour after, IV injection of L-DOPA (15 mg/Kg). Average unit activity at each temperature was determined and periussial time histograms were constructed by a PDP-11 computer. The following groups of acute spinal animals were used: a) no pretreatment; b) p-chlorophenylalanine (PCPA) (a 5-HT depletor); c) 6-hydroxydopamine (6-OHDA) (twice intracisternally); d) fusaric acid (FA) (dopamine-β-hydroxylase inhibitor). 

L-DOPA caused marked inhibition of the unit response to noxious thermal stimulation (maximum average inhibition was to 35% of the control response). This inhibition was dependent on intact 5-HT stores and was not abolished by destruction of catecholamine tracts by 6-OHDA or by blocking the conversion of dopamine to NA by fusaric acid. 

The results suggest that NA alone has minimal inhibitory effect on dorsal horn nociceptive units. Most of the inhibition could be ascribed to L-DOPA triggered 5-HT release. Thus direct NA influences on segmental nociceptive mechanisms appear to be minimal, and previously reported inhibition (Anden et al., Acta Physiol. Scand. 67:373, 1966; Pearson, Proc. Soc. Neurosci. 14570, 1978) is possibly caused by noradrenergic interactions with serotonergic or other descending systems.
AXON COLLATERALS OF DORSAL HORN CELLS RESPONDING TO CUTANEOUS STIMULATION  

Intracellular injections of horseradish peroxidase into dorsal horn cells in lumbar segments of the cat's spinal cord revealed distinctive patterns of axonal projections from cells responding to low threshold cutaneous stimulation of the hind foot. Axonal projections have been examined in lamina IV, 10 in lamina IV, 4 in lamina V, and 3 in lamina VI. In general, cells responding to stimulation of the central foot pad were located most medially, those responding to stimulation of the proximal foot and dorsal surface of the toes were most lateral.

A striking characteristic of the axonal projections of many of these dorsal horn cells was a dense arborization of collaterals in the laminar region ventral to the cell body.

In addition, all cells contributed axons to the ipsilateral white matter, many with two separate branches that either both ascended, or ascended and descended within the same white column, or entered entirely separate columns. In lamina IV, two cells that contributed axons to the dorsolateral funiculus were probably cells of the spinocervical tract. Of the other 8, the 3 most medial contributed fibers to the medial dorsal column, the 3 most lateral contributed fibers to the lateral column or to the lateral fascicles of the dorsal intracolumnar tract (DIT). One of these also sent an axon collateral to the dorsal portion of the dorsal column. Of the two lamina IV cells lying in an intermediate position, one sent a collateral to the medial dorsal column, the other to the lateral portion of the DIT, as well as a collateral to the dorsal portion of the dorsal column. This same medial-lateral organization of axon collaterals tended to hold for the 4 cells of lamina V, but in lamina VI, all 3 cells, including two quite medially placed, sent long axon collaterals to the lateral column only.

In summary, 1) medial cells in lamina IV tended to contribute axons to the medial dorsal column, while lateral cells in lamina IV tended to send axons to the lateral column, and 2) most of the dorsal horn cells studied had, in addition to at least one long projection axon, from one to 4 collaterals arising from the main axon and distributing within the dorsal horn in approximately the same transverse level as the cell body. The pattern of distribution of these collaterals varied widely, but a prominent feature was that the collaterals were distributed ventrally to the cell body, often most heavily in the lamina just ventral to the on in which the cell body was found.

(Supported by grants from NIMH and NSF.)

CHARACTERISTICS OF INDIVIDUAL MEDIAL GASTROCNEMIUS Ia AFFERENT PROJECTIONS TO TYPE-IDENTIFIED TRICEPS SURAE MOTONEURONS  

Motoneurons may be divided into more or less discrete motor unit groups based on the properties of the muscle fibers they innervate. Burke and colleagues (see J. Neurophysiol. 39: 447, 1976) have shown that a single input to a single triceps surae motoneuron (TSM) exerts differential effects as a function of motor unit type. We are studying the projection frequencies of individual Ia afferent fibers and the characteristics of single fiber Ia EPSPs in type-identified TSMs using the spike-triggered averaging technique.

TSM intracellular potentials, obtained by conventional techniques, are led to a signal analyzer, which is triggered by action potentials in a single medial gastrocnemius Ia fiber. The EPSP generated from a single fiber's terminals is accumulated by the analyzer. Intracellular current pulses are then injected to generate specific discharge patterns in the TSM. Resulting single and repetitive muscle unit contractions are measured with a strain gauge. Motor units are classified as FF, FR, or S based on the shape of an unfused tateus tension envelope and fatigue resistance, using Burke's criteria.

To date, EPSPs have been studied in 45 type-identified TSMs in 4 cats. Although the sample size makes statistical evaluation inappropriate, these preliminary data show a trend toward higher projection frequency and larger single-fiber EPSPs in FR units than S units. More detailed analysis will require a considerable increase in sample size. Characteristics of the single fiber projection will be discussed in relation to data obtained with whole nerve stimulation.

ANTEROGRADE TRANSPORT OF HORSE RADISH PEROXIDASE IN THE PROG SPINAL CORD  
F. Glanzman*, Patricia L. Mensah, Dennis Glanzman and Richard P. Thompson. Department of Psychology, Arizona State University, Tempe, AZ; Department of Anatomy, School of Medicine, University of Southern California, Los Angeles, CA; and Department of Psychobiology, University of California, Irvine, CA.

Twenty adult frogs (Rana catesbeiana) measuring three to four inches in length were used in this study. Horseradish peroxidase (Sigma type VI) was injected into caudal medulla or cervical, thoracic and lumbar spinal cord. Large injections were accomplished under MS 222 (Tricaine) anesthesia by delivering 0.4 to 0.6 microliters of a 20% solution of HRP dissolved in distilled water. After a survival period of one to four days at room temperature, the animals were perfused intracardially with a fixative containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer. The brain and spinal cord were then removed, post-fixed in the same fixative for 3 hours, stored in 0.1M phosphate buffer and 5% sucrose for 3 days, and sectioned at 40 micra. Sections were washed in buffer and processed for the blue reaction product according to the technique described by Mesulam (J. Histocherm. Cytochern., 24: 1273, 1976). Sections were counterstained with 1% neutral red. Significant amounts of anterograde transport occurred at all times examined. After caudal medullas injections, spinal projecting fibers were seen in the lateral funiculus and in the area of the ventral funiculus occupied by the vestibulospinal system. These fibers entered the central gray and established terminal fields as described previously in lesioned Pkx-Heimer stained material by Mensah and Thompson (J. Anat., 125: 1, 1978) for the lateral column fibers and by Orozko and Dosiova (Prog. Brain Res. 37: 297, 1972) for the vestibulospinal system. Extensive pericellular networks were common and bouton-like enlargements were seen along the course of individual axons. Those results indicate that anterograde transport did occur over long distances. In addition, this study suggests that HRP histochemistry may be a reliable method for marking the terminal endigations of individual fiber systems. (Supported by NIMH grant number MH-25127 to R.F.T.)

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Although several studies on the CNS distribution of immunoreactive leucine-enkephalin, there are no detailed descriptions of the location and morphology of enkephalin somata. In the present study enkephalin perikarya in the spinal cord were studied by immunocytochemistry. The cats were administered either an intraventricular (100µg in 5 µl) or an intraspinal (100µg in 2 µl) injection of a horseradish peroxidase (HRP) that overlapped with the dendritic fields of the phrenic neurons. Such a sustained negative shift of the potential of fibers in an adjacent DR. Such a sustained potential was consistently succeeded by a sustained vestibulospinal reflex (MSR) function in the cat, positive DRPs—which indicate hyperpolarization of adjacent terminals—have been recorded from cervical roots (DR) following single volleys in adjacent DRs this has been attributed to the ready releasable pool in the spinal cord. Further, SOL and MG appear to be differentially sensitive to the neurotoxic actions of acrylamide. Supported by NS-11948.


The motoneurones which innervate the diaphragm of the adult rat were localized and morphologically characterized in the spinal cord by the method for the retrograde transport of horseradish peroxidase and the zinc chromatographic modification of the Golgi technique. These neurones were distinguished from those in the cervical and thoracic segments by the fact that most of the diaphragm and those which give rise to the accessory phrenic nerve, peroxidase was applied to crushed phrenic axons either in the cervical or thoracic segments. The distribution of these neurones in the cervical segments of the spinal cord in sagittal sections pinholes were inserted perpendicularly to the long axis of the cord through the center of the C3-C7 dorsal roots to identify spinal cord levels. The spinal cord was notched at C2 to distinguish rostral from caudal ends of each section. When peroxidase was applied in the neck, labelled cells were found at the C3-C6 levels of the spinal cord. However, when the application of peroxidase included accessory phrenic fibers, labelled cells were found at the C3-C6 levels of the cord. The labelled neurones were mostly polygonal or fusiform in shape and peroxidase activity ranged from lightly labelled to heavily labelled cells. The long axes of the heavily labelled cell bodies ranged from 32-46 µm while the short axes ranged from 19-26 µm. In most cases clusters of 2-6 closely apposed cell bodies were observed, but isolated cells were occasionally found. In frontal sections the location of peroxidase labelled cell bodies was determined from measurements along mid-sagittal and horizontal coordinates using the center of the spinal canal as a zero reference point. The greatest number of labelled cells were seen at C4. At this level labelled somata occupied a cross-sectional area of approximately 150 µm in diameter with the center of the area lying 600 µm lateral to the mid-sagittal plane and 400 µm anterior to the horizontal plane. The area of the center of the collapsed rostrolaterally at C3 and anteromedially at C5 and C6. Phrenic motoneurones were identified from rostral to caudal sections using the above coordinate system. Examination of Golgi sections showed that dendritic fields of neurones were several thick dendrites which branched repeatedly and radiated in the lateral and ventral grey. These neurones were located at the periphery of the larger phrenic cell bodies. These small neurones displayed delicate, beaded dendrites which overlapped with the dendritic fields of other adjacent motoneurones. Dendritic overlap between phrenic neurones and other adjacent neurones also occurred. Supported by the Paralyzed Veterans of America and NIMH grant NS 06925-13.
GLUCOCORTICOID INTERACTIONS WITH BIOGENIC AMINE AGONISTS AND ANTAGONISTS ON CAT SPINAL REFLEXES. Edward D. Hall, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

A short-term triamcinolone diacetate regimen (8 mg/kg i.m./7 days) has been demonstrated in acute spinal (C-1) cats to enhance excitatory spinal monoaminergic (5HT) postsynaptic reflex actions (Hall et al., J. Pharmacol. Exp. Ther. 206:361, 1978; Hall and Baker, J. Pharmacol. Exp. Ther. In press). In the present work, the effects of chronic triamcinolone dosing have been examined on the 2N actions of alpha adrenergic and serotoninergic agonists and antagonists. In all experiments, 1.2X supramaximal stimuli were applied to the typical anterolateral nerves of one leg every 5 sec. and the evoked reflex responses recorded at the ipsilateral L7 or S1 ventral root. In some series of experiments, the 2N reflex effects of a 1 mg/kg dose of the centrally-active noradrenergic agonist methoxamine (OX) given as a 20 minute infusion were studied. As described by Vaugel and Martin (J. Pharmacol. Exp. Ther. 195:87, 1976), Minoxidil (MX) produced a progressive increase in the 2N response amplitude in untreated cats with a maximum of 34.5%. In the glucocorticoid treated animals, however, no increase by MX was seen; rather a 10-15% depression in response amplitude occurred. One hour after the MX infusion, 20 mg/kg phenoxbenzamine (PBZ) was infused over 20 minutes. In the untreated cats, alpha adrenergic receptor blockade by PBZ produced a 24.2% decrease in the 2N response while in the treated animals, PBZ caused an increase of 21.4X.

In a second series of experiments, the experimental protocol of Clineschmidt et al (J. Pharmacol. Exp. Ther. 179:312, 1971) was employed to assess glucocorticoid effects on the 2N reflex actions of serotonin manipulation. Fifty mg/kg 5-hydroxytryptophan (5-HTP) was infused over 10 min.; followed at 60 min. by 1 mg/kg amitriptyline (AMT) over 5 min.; followed 25 min. later by 1 mg/kg methysergide (MS) over 5 min. In the untreated animals, 5-HTP produced a moderate depression (20% of control) during AMT. When serotonin reuptake inhibitor AMT was then given, an immediate elevation (40-50% above control) in the response was observed which was rapidly diminished by the subsequent administration of the serotonin antagonist MS. In the treated preparations, 5-HTP produced the same 2N depression, but subsequent AMT produced a 3-fold greater increase in the response that was refractory to antagonism by MS.

These data may have relevance to the pathophysiology of psychotropic depression in man. For instance, in many individuals with depression, there is a significant elevation in plasma levels of the endogenous glucocorticoid cortisol (Prange et al., Life Sci. 20:1305, 1977) which could as the present results suggest, have a significant effect on central biogenic amine transmission. (Supported by NIMH Small Grant MH 31887-01).


Extra spinal neural by-pass (ESNB) was performed in the right hand limb of 20 rats subjected to spinal cord transection. The ESNB utilized a graft from the right ilioinguinal nerves to the right sciatic nerve (see figure below). Three months post-operatively, discrete flexor movements of the toes were elicited by stretch evoked impulses in the endogenous glucocorticoid cortisol (Prange et al., Life Sci. 20:1305, 1977) but also a localized depression, there is a significant elevation in plasma levels of the endogenous glucocorticoid cortisol (Prange et al., Life Sci. 20:1305, 1977) which could as the present results suggest, have a significant effect on central biogenic amine transmission. (Supported by NIMH Small Grant MH 31887-01).

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Motor apparatus. It was subsequently shown that the discharge of 40% and 56% of the extensor MNs studied during controlled treadmill locomotion (evoked by brainstem stimulation) was characterized by an initial short interburst interval (ISI) of 120 ± 26 msec, while the intervals separating the spikes in the remainder of the burst were longer and relatively constant (Rajak and Young, 1979). It was suggested that this discharge pattern reflected the most efficient method of maximizing tension production. Evidence is now available (R.B. Smith, personal communication) that the parameters of MN discharge which maximize the force per impulse is one in which a short initial ISI is followed by a relatively long ISI with the remaining ISIs being either of the same magnitude or constant.

The discharge of MNs which were active during controlled treadmill locomotion or fictive locomotion was analysed to determine whether the pattern which has been derived experimentally in one series of experiments, cats were decerebrated and walked on a treadmill in response to short trains of electric shocks to the sacral spinal cord segments containing the labeled cells. It was determined that the particular techniques employed in this study yielded results which were in agreement with those of previous experiments in which some MNs were found to fire during both types of locomotion. The mean initial ISI seen in MNs active during treadmill locomotion was much shorter (15 ± 2 msec) than that observed during fictive locomotion (29 ± 22 msec). With one exception, MNs which produced less than 10 spikes/burst did not display an initial doublet, whereas the mean initial ISI for those with more than 10 spikes/burst was 11.2 msec. These data suggest that the details of the firing patterns during locomotion are centrally determined. (Supported by M.R.C. of Canada)

Although some MNs were found to fire during both types of locomotion according to the pattern proposed by Stein, the pattern most commonly observed consisted of a fairly regularly spaced spike train with mean ISIs in the range of 40 - 50 msec, with a shorter initial ISI (mean = 15 ± 2 msec) and a relatively long ISI with the remaining ISIs being either of the same magnitude or constant.

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HOW THE SIZE OF MOTONEURONS DETERMINES THEIR SUSCEPTIBILITY TO DISCHARGE. Hans-R. Lüscher*1, Paul Ruenzel*2 and Elwood Henneman. Dept. of Physiology, Harvard Medical School, Boston.

Vessels of Ia impulses evoke aggregate EPSPs in motoneurons (MNs), whose amplitudes correlate inversely with cell size and directly with susceptibility to discharge. The following hypothesis is proposed to explain the basis for these relationships. (1) Ia fibers approaching a MN branch more frequently and send more terminals to cells with larger surface areas; (2) the more branching there is, the greater is the possibility that impulses in these fibers will fail to invade all the terminals; (3) the percentage of synaptic knobs activated by Ia impulses is, therefore, determined by the branching pattern in the terminal arborization and by factors that influence invasion of terminals. To test this hypothesis, the sizes of aggregate EPSPs in MNs before and after post-tetanic potentiation (PTP) were related to the input resistance (IR) of the cells, which was measured by the "spike height" method. Ventral roots of L1 and S2 were severed to eliminate backfiring of MNs, which interferes with recordings of EPSPs. Hindlimb muscle nerves in cats were stimulated at 1/sec with shocks that set up small volley in all their Ia fibers. A series of 16 mono-synaptic aggregate EPSPs was recorded from the MN and averaged electronically. The muscle nerve was then tetanized with similar shocks at 500/sec for 10 sec. Thereafter another series of 16 EPSPs was recorded and averaged. As our hypothesis predicts, the small control EPSPs and the greatest PTP potentiations occurred in MNs with the lowest IRs, i.e., in the largest cells. It has been assumed that PTP is due to release of more transmitter from a fixed number of active endings. This theory predicts that the magnitude of PTP is independent of cell size and not apparent with few exceptions. PTP increased the rise-times and half widths of EPSPs, effects consistent with invasion of additional terminals. The findings suggest that invasion of Ia terminals is a graded process that is more complete in arborizations on small cells, which explains their greater susceptibility to discharge.

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CONDUCTION STUDIES IN ISOLATED PERFUSED SPINAL CORD AND IMPLICATIONS FOR THE CAUSES OF IRREVERSIBLE SPINAL INJURY. H.F. Martin, S. Katz, and J.G. Blackburn. Department of Physiology, Medical University of South Carolina, Charleston, South Carolina 29403.

Irreversible effects of spinal cord trauma are thought to be due to either direct damage to neural structures (i.e. membrane disruption) or to necrosis secondary to anoxia following microvascular injury. The latter hypothesis has been investigated by subjecting the spinal cord to anoxia separate from trauma. We have developed a model to investigate the former hypothesis by allowing traumatic damage without anoxia.

Excised spinal cord strips from cats or rats were placed in a chamber perfused by oxygenated Ringer's solution. Stimulating and recording electrodes were positioned to measure axonal conduction along the length of the strip. Results of these experiments have shown that conduction can be maintained for an excess of four hours provided adequately oxygenated fluid remains in contact with the tissue. Raising the cord segment into a layer of mineral oil attenuates conduction within 30 minutes, however, conduction can be restored by renewed perfusion by oxygenated Ringer's solution.

This model forms the basis for continuing investigations into the temporal sequence of changes in neural conduction following trauma. (Supported by NINDS grant #P-SP73-NS-11066).

EVIDENCE FOR FOUR DIRECT BRAINSTEM PROJECTIONS TO THE INTERMEDIATE-LATERAL CELL COLUMN WITH NOTES ON THEIR DEVELOPMENT. George F. Martin, Dept. Anat., Sch. Med., The Ohio State University, Columbus, Ohio, 43210.

The horseradish peroxidase method was used to identify those areas of the brainstem which project to the thoracic cord and would thus be possible sources for direct projections to the intermedullary cell column. Injections of H-2-leucine were made into all of the areas identified by the HRP technique. The resulting autoradiograms show that the intermedullary cell column is innervated by at least four areas. Injections which include the nucleus cuneolus (CoG) and closely adjacent areas elicit labeling of the nucleus intercalatus that lie laterally in the intermedullary cell column. Bilateral labeling of the same region is present after injections of the nucleus reticularis gigantocellularis pars ventralis (Rgcv), pars lateralis (Rgcl), and nucleus raphe medianus and magnus (Rm). Since fluorescent varicosities of both types pack the intermedullary cell column and it is known that they arise within suprasegmental centers, it seems reasonable to conclude that they take origin from the areas referred to above. Developmental studies of ponceau-yellow opossums show that fluorescence appears in sympathetic ganglia of the peripheral nervous systems very early (11 days after conception) and that the intermedullary cell column is one of the first targets of brainstem neurites. Supported by U.S.P.H.S. Grants NS-07410 and NS-10165.


The differential effects of compression & ischemia on nerve fibers have been incompletely studied. Most data has been derived from work on the peripheral nervous system. To determine the effect of experimental spinal cord compression injury on feline spinal cord white matter, fiber densities & the fiber spectrum (diameter distribution) was computed for the dorsal column of normal & injured animals. Adult, female, mongrel cats were subjected to laminectomy at the upper lumbar area & the wound was either closed or the spinal cord was compressed using a 170gm wt for 5 minutes. At 30, 45 & 60 days post-compression, the animals were sacrificed under pentobarbital anesthesia by intracardiac perfusion fixation using buffered 5% gluteraldehyde. Transverse sections of spinal cord were cut at 1 micron, stained with toluidine blue & mounted on glass slides. Using a Zeiss photomicroscope, every 6th microscopic field was systematically sampled. The fiber diameter distribution was then computed from the photographic enlargements using a log-arithmic mode. The dorsal columns of normal (laminectomy alone) spinal cord contained from 39000 to 56000 myelinated fibers/mm². Fiber diameters ranged from 0.5-15 micra, the majority falling in the 2-5 micron range. Injured animals showed an average fiber density in the dorsal columns of 8000 myelinated fibers/mm². Myelinated fibers of all diameters showed a reduction in number but there was a statistically significant differential loss of fibers in the 2-4 micron range. Previous studies on peripheral nerve regeneration show that a large loss of small myelinated fibers to compression injury while smaller myelinated fibers have been more susceptible to ischemia. The pathogenesis of experimental spinal cord trauma is unclear but ischemia plays a significant role in the auto-destructive process following blunt trauma. Although fibers were decreased over the entire fiber spectrum, the differential loss of small myelinated fibers suggests that ischemia may play a more significant role than compression in experimental spinal cord injury. Moreover, the absence of total absence of degenerating fibers at the C3 level at 60 days post-compression suggests that Wallerian degeneration was complete by this time. Supported in part by the VA & a grant from the Paralyzed Veterans of America & The Upjohn Co.

Spontaneous, negative-going, slow waves are the characteristic components of the spontaneous spinal electrical signal (SEG) as recorded from the dorsal root ganglia in the intact spinal cord. It has been demonstrated that spontaneous slow waves increase in frequency of occurrence in the intact cord caudal to a site subjected to blunt trauma. The degree of laminectomy is not a factor, since slow waves appear to correlate well with the extent of the lesion that results from blunt trauma. Because of this the SEG may be an important diagnostic tool in the evaluation of the spinal cord, and in the determination of effectiveness of treatment modalities. It has been shown by mapping the amplitude of slow waves as a function of depth within the cord that neurons in the dorsal gray matter may be a source of the waves. This experiment was designed to establish the cellular source of the slow potentials.

In anesthetized, decerebrate cats with a spinal transaction at the T12 level, slow waves were recorded from a silver ball electrode on the exposed surface of the cord 2mm lateral to the midline at the L6 segment. Less than 1mm from this site a microelectrode was inserted into the cord and the spontaneous discharge of single neurons were recorded. Many neurons were sampled within a dorsal-ventral track through the gray matter, while simultaneously recording the SEG. The finding that these units had time intervals that show a Poisson distribution. However, in all tracks cells were encountered that showed histogram with definite bimodality. Cross correlation analysis indicates that firing in these cells correlate with the occurrence of slow waves. Cross expectation density histograms show the correlation to occur with periods less than the slow wave and in some cases the slow waves or the units are treated as the generating source. Electrolytic lesions placed at sites where non-Poison unit activity was observed show these units to be located in lamina IV of the dorsal horn.

These results suggest that a population of units in the dorsal gray matter show spontaneous activity that is reflected in a surface slow wave. That the occurrence of the slow wave can suddenly precede, coincide with, or slightly follow the occurrence of unit firings indicates that the slow wave may result from summated excitatory post-synaptic potentials in these cells. Supported by NINCDS grant 13042.

IMMUNOFLUORESCENCE STUDIES WITH GFA AND NEUROFILAMENT ANTISERA IN RATS WITH SPINAL CORD TRACTION. Chi Nguyen , Rotte Buhl, Rich Nguyen* and Amico Bignami. Spinal Cord Injury Service, West Roxbury Veterans Administration Medical Center and Harvard Medical School, Boston, Mass. 02132.

Supported on the National Institute of Neurological and Communicative Diseases and Stroke grant NS 12751 and by USPHS Grant NS 13034 and by the Veterans Administration.

Although the peripheral contributions of the phrenic nerve in the guinea pig (C4, C5, C6) have been documented by Cooper and Schiller (1975), knowledge regarding its central connections is limited. The purpose of this study was to determine the body of information that exists for other mammals, including man, that suggests the phrenic nucleus constitutes a discrete, intermediately placed column of cells in the ventral horn. The longitudinal distribution has been observed rostrally to the third and caudally to the seventh cervical segments. Furthermore, the longitudinal placement of motor neurons does not always correspond to the ventral rami contributing to the phrenic nerve (Ullah, 1978). The ratio of afferent to efferent fibers of the phrenic nerve has not been reported in the guinea pig.

The present study exploited the HRP technique to segmentally localize the sensory and motor cell bodies giving rise to the phrenic nerve. Thoracotomies were performed anesthetically on animals anesthetized with a combination of chloral hydrate and Inovar. HRP pellets were placed on crushed portions of the exposed right phrenic nerve and the enzyme isolated from the adjacent tissues by a silicium cuff. Allowing sufficient time for HRP transport, the animals were sacrificed and the cervical cord along with the right dorsal root ganglia were removed. A stereotaxic technique followed Mesulam's technique. Longitudinal and transverse sections were made defining rostral to caudal and medial to lateral extensions of the motoneuron cell bodies. The number of cells stained in the dorsal root ganglia were counted and segmentally defined.

The present data indicate a longitudinal distribution of the motor cell bodies extends further rostrally than the previously reported peripheral contributions would suggest. The morphological aspects of this study will be discussed in connection with data existing from other mammals. (Supported by the Veterans Administration and NIH Grant NS14431-02).

SPINAL CORD AMINO ACID LEVELS IN NORMAL AND DIABETIC RATS. James T. Patrick*, David L. Felten, Michael A. Rae*, and William J. McBride (Support: National J.P.). Department of Anatomy, Psychiatry and Biochemistry and Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

Levels of aminobutyrate (GABA), aspartate (Asp) and glutamate (Glu) were determined in thoracic spinal cord gray areas of normal and streptozotocin-induced diabetic rats. Harvesting a disarticulated microscope mounted on the outside of a specially constructed cold-box, microdisks of tissue were punched out from the dorsal gray (DG), intermediate gray (IG) and ventral gray (VG) using a modified needle (i.d. 330µm) with styllet. In the spinal cords from normal rats, an uneven distribution of amino acids was observed (Table): the level of GABA was significantly higher in the DG than in the IG and VG; the levels of Asp were significantly higher in the IG and VG then in the DG; the level of Glu was significantly higher in the IG than in the DG and VG; although there was no significant difference in the levels of glycine, there appeared to be a trend towards higher levels in the VG and IG.

### Table: Distribution of Amino Acids in Rat Thoracic Spinal Cord

<table>
<thead>
<tr>
<th>Area</th>
<th>Ala</th>
<th>Gly</th>
<th>GABA</th>
<th>Asp</th>
<th>Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG</td>
<td>7.4±0.9</td>
<td>48.7±1.8</td>
<td>18.1±0.8</td>
<td>22.9±2.7</td>
<td>85.1±1.9</td>
</tr>
<tr>
<td>IG</td>
<td>6.8±0.7</td>
<td>67.6±1.1</td>
<td>12.2±2.7</td>
<td>34.2±2.7</td>
<td>103.1±2.7</td>
</tr>
<tr>
<td>VG</td>
<td>5.9±0.4</td>
<td>64.6±1.4</td>
<td>6.2±0.5</td>
<td>32.4±0.9</td>
<td>80.7±2.8</td>
</tr>
</tbody>
</table>

Levels of amino acids are expressed as nanomoles/mg protein ± SE; significance (p<0.05) for DG vs IG (*), DG vs VG (**), and DG vs IG (***)

In the diabetic rats, neuronal counts revealed a decrease of alpha motoneurons by 42% in the VG and a decrease of interneurons by 11% in the IG and VG (D.L. Felten, Clin. Res. 26: 67A, 1978). A comparison of the levels of the five amino acids in the DG, VG and IG of normal and diabetic rats showed no significant difference. This suggests a nonselective diabetogenic loss of neurons. (Supported by NIMH Grant P60 AM 20542 and Alfred P. Sloan Foundation Fellowship (MLP)).


Physiologically identified axons from cutaneous high threshold (nociceptive) and "D-hair" mechanoreceptors of cat were stained intracellularly by lontophoresis of horseradish peroxidase. Histochemical reaction produced an electron-dense staining of the fibers and their terminations, traceable within the spinal dorsal horn. Ultra-thin serial sections were made of axonal branches selected by light microscopy.

Synaptic enlargements of high threshold mechanoreceptors are found principally in the ipsilateral marginal zone and the neck of the dorsal horn; boutons in the marginal zone contain round synaptic vesicles and, occasionally, dense-core vesicles (90 to 150 nm in diameter). The terminations often partially enclose dendritic processes, are filled with clear, round vesicles. The boutons make synaptic contacts with several dendritic structures. Some "D-hair" boutons are contacted by profiles containing flattened vesicles.

Thus, even though "D-hair" and high threshold mechanoreceptors have axons with overlapping (slow) conduction velocities, they differ not only in their regions of termination, but also in the morphology and ultrastructure of their central synaptic terminations.

Supported by Grants NS10321, NS16899, and NSF05576 from the National Institute of Neurological and Communicative Disorders and Stroke of the United States Public Health Service; and aided by an exchange agreement with the First and Second Departments of Anatomy, Semmelweis University Medical School, Budapest, under the United States-Hungary Program of the National Science Foundation.
Detection of Sub-clinical Spinal Tract Sensory Dysfunction.

Richard J. Schneider and Ronald Burke*.

The ability of humans and rhesus monkeys to discriminate between two somatosensory stimuli was examined in the context of signal detection theory (TSD). The subjects were seated with one leg restrained as a discrete group of hair shafts were stimulated in an oscillatory fashion. Two different frequencies of stimulation were used. The discrimination task required that the subject push a button in response to the "go" stimulus and refrain from pushing it in response to the "no-go" stimulus. Response latencies were recorded in 100 msec. time bins which formed the basis of a confidence rating scale and allowed the sensitivity of each subject to be characterized in the form of a receiver operating curve (ROC). A population of normal human subjects was found to generate reliable ROCs with high sensitivity to phrenic nerve stimulation. However, the literature is noticeably lacking in conclusive evidence of the exact segmental localization and extent of the phrenic nerve. There appears to be almost complete agreement that the phrenic nerve cells lie in the fourth cervical segment, irrespective of species (Warwick et al.).

In the past we determined the distribution of these cells bodies further extended thru the fifth and sixth cervical segments in dogs. In regard to the ratio of affe­rent to efferent fibers, Landau, using the defascerted dog, found that efferent fibers comprised 55-65% of the fibers supplying the phrenic nerve. We conclude that TSD may be utilized to produce reproducibly labelled neurons deep in the intermediate gray zone and ventral horn of the spinal cord between C1 and C6. Moreover, a varying discriminative ability, from adequate to extremely acute was shown. There was some evidence that younger subjects made the discrimination with greater facility than older ones. The results of the present study are thus consistent in human populations. Moreover, as motor fatigue is well-documented in MS, there may be a sensory nervous system fatigue rather than by discrimination functions as the test went on. Finally, we believe the first threshold to be related exclusively to hair-follicle stimulation and from our previous studies that indicates a possible functional ability. The second threshold is a well-defined threshold which reflects the ability to make the discrimination even when the subject is explicitly informed of the task at hand. In conclusion, the results of the present study support the hypothesis that sensory discriminations may influence the detection of peripheral nerve lesions in the setting of multiple sclerosis.

Supported by Research Grant 80-1207-A-1 from the National Multiple Sclerosis Society.

It has been reported that [Ca2+]o declines during stimulation and during seizures in cerebral cortex, cerebellar cortex and sympathetic ganglia. I used Ca-selective microelectrodes to measure [Ca2+]o, in gray matter of unanesthetized decapitate spinal cords. Electrical C.E. was recorded from the barrel reference of the microelectrode. VRS were cut and the sciatic nerve stimulated repetitively. Changes of [Ca2+]o were small and often not detectable (ref. 1).

Some preparations repetitive stimulation was consistently associated with decreases of [Ca2+]o. Sustained potentiation (SP) was, however, present when stimulation was continued beyond 20 min. In some preparations repetitive stimulation was associated with increases of [Ca2+]o. The timecourse of [Ca2+]o was slower than that of SP, and the magnitude of (Ca2+) was much smaller than that of SP. The difference cannot be explained by different concentrations of post-synaptic potential.
EFFECTS OF L-DOPA ON DORSAL HORN UNIT RESPONSES TO INNOCUOUS STIMULATION FOLLOWING PRETREATMENT WITH (1) 6-OHDA (2) PUSARIC ACID. Charles I. Woods*, Charles J. Hodge and Jonathan Delatizky. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Studies of L-DOPA effects on sensory transmission suggest that L-DOPA modulates sensory input by releasing noradrenaline (NA) from noradrenergic nerve terminals in the spinal cord. Descending noradrenergic pathways from the locus coeruleus and other pontomedullary reticular areas are the only source of spinal cord NA. In a previous study we demonstrated that L-DOPA increases responsiveness of cells which respond only to innocuous stimuli, and depresses the activity of cells which respond to noxious stimuli, and showed that the inhibitory effects of L-DOPA are abolished by pretreatment with the serotonin depletor, p-chlorophenylalanine (PCPA). The present study was undertaken to determine if the effects of L-DOPA are specifically dependent on intact bulbospinal noradrenergic pathways and if these effects are due to NA rather than other metabolites of L-DOPA.

6-hydroxydopamine (6-OHDA), which causes selective degeneration of catecholamine fibers, was administered intracerebrally to one group of animals (Breese & Taylor, Br. J. Pharm. 62:88, 1971), and fusaric acid, an inhibitor of dopamine-B-hydroxylase, was administered to another group (Nagatsu et al., Biochem. Pharmac. 19:35, 1970).

RESULTS AND DISCUSSION: Group I cells showed no change or a small decrease in responsiveness, (2) Fusaric acid pretreatment: (only group I cells were studied), most cells showed no change or a decrease in responsiveness. The results suggest (1) the facilitatory effects of L-DOPA are specifically dependent on intact bulbospinal noradrenergic pathways, (2) the inhibitory effects caused by overflow of 5-HT are not mediated through noradrenergic pathways. The results of the fusaric acid experiments suggest that the facilitatory effects of L-DOPA are due to release of NA and not other L-DOPA metabolites, such as dopamine.

FUNCTIONAL CHARACTERISTICS OF LARGE AND SMALL NEURONS IN SUPERFICIAL LAMINAE ([I-III] OF THE CAT DORSAL HORN. Robert P. Yezierski, Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Adequate stimulus and receptive field (RF) characteristics were determined for single neurons in laminae I-III of urethane anesthetized, spinal cats. Units were characterized while tracking small amplitude changes of waveforms with commonly used functional properties using a real-time waveform recognition technique previously described (Yezierski et al., 1978). Locations of maximum spike amplitudes were marked with ferric ions and Prussian-blue dots reconstructed using camera lucida. Recordings were obtained from neurons across the full mediolateral extent of the gray in L6 and L7. Data from amplitude profiles were used in calculating source voltages; these voltages were used as an index to approximate size of neurons from which recordings were made. Results of this study confirm anatomical findings that neurons in these laminae constitute a heterogeneous population with respect to size. Seventy-five percent of the neurons in laminae I-III had source voltages in the smallest calculated range; 30% of the neurons in deeper laminae were found in this range. Forty-two percent of the intermediate to large cells in laminae I-III were driven by light touch and noxious cutaneous stimulation; seven percent were driven only by noxious stimuli. Nineteen percent of the small neurons were driven by light touch, pressure, temperature, and noxious innocuous (NI) responses, respectively. The relationships between RF characteristics (length/width ratios, RF area) and distance from toes were similar for both large and small neurons to those reported in deeper laminae (Brown et al., 1973). This research was supported by USPHS grant NS12061 awarded to P. B. Brown.


The syndrome "tethered cord" has been recently recognized as a neurological entity in children manifesting progressive signs, such as: incontinence and motor and sensory changes in the legs. This disorder is often associated with spinal anomalies and an elongated spinal cord. Neurosurgeons have experienced that untethering of the spinal cord often results in improvement of neurological signs.

Since neuronal cells rely absolutely on energy derived from intramitochondrial ADP phosphorylation, we postulated that oxidative metabolism of the tethered cord may be reduced below a critical level, thereby resulting in progressive neuronal injury. We applied the dual wave-length reflection spectrophotometer to the experimental tethered cord in vivo, and monitored changes in the reduction/oxidation ratio of cytochrome aa3, the terminal oxidase of the respiratory chain.

Twenty cats were anesthetized initially with ketamine and then with N2O and O2, and the lumbar sacral spinal cord was exposed. Optical measurements were made at the junction of the lumbar and sacral cord. Tethering was produced by traction applied with 2-0 silk suture, one end tied around the filum terminale, the other passed over a pulley and attached to weights (1-3g). In addition, the animals were subjected to anoxic stress with 1000 N2O inhalation for 2.5 min. Spinal cord potentials were taken from its dorsal surface in response to the L6 nerve root.

RESULTS: (1) The Traction Without Anoxic Stress: No significant changes occurred in redox level of cytochrome aa3, with traction 1-3g, whereas 5g traction resulted in a mild reduction. However, this reduction level is far less than the maximum reduction level obtained by anoxia.

(2) Traction Under Anoxic Stress: As traction weight was increased, increases in the reduction level of cytochrome aa3 during anoxia became slow. Under 4 or 5g traction, changes in reduction levels failed to reach the maximum obtained with traction 0-3g; the neuron potentials failed more rapidly under 3g traction than under 5-9g traction.

CONCLUSION: The experimental cord with 5g traction responds to anoxia with only minimum redox change. We believe the spinal cord under this circumstance resembles traumatized or anoxic cord with cytochrome aa3 reduced more rapidly due to vascular insufficiency. Untethering improves the oxidative metabolism of the cord, if neuronal mitochondria are not irreversibly damaged.
SYNAPTIC TRANSMISSION
A NORADRENERGIC S-IPSP IN MAMMALIAN SYMPATHETIC GANGLION. John H. Ashe and B. Libet, Department of Physiology, School of Medicine, University of California, San Francisco, CA 94143.

The atropinized superior cervical ganglion of the rabbit at 22°C exhibits a surface-positive (hyperpolarizing) potential, lasting 1–2 min, during and following a train of ganglionic spikes elicited by preganglionic nerve volleys (e.g., 5/sec for 8 sec). A consideration of the hyperpolarizing phase removed by incubation (60–90 min) with the alpha-blocker phenoxybenzamine (9 μM–12 μM), but not with the β-blocker propranolol (10 μM), indicates the apparent contribution of an adrenergic component. The remaining hyperpolarization is then “pure” posttetanic hyperpolarization (PT-HP), due to activity of the sodium potassium pump (Sanchez et al., Life Sci. 20: 1863–1870, 1977). PT-HP recorded at non-synaptic postganglionic axons is not affected by phenoxybenzamine.

The apparently adrenergic component of the hyperpolarization can also be eliminated by incubation with methacholine. This loss is presumably due to cholinergic action on an intraganglionic presynaptic store of catecholamine resulting in excessive release and depolarization; subsequent incubation with NE or DA can in fact restore the hyperpolarization, with NE more effective. This contrasts with muscarinic depletion of VIP cells which is resistant to atropine, in which only DA is effective for restoration (Libet and U.S.P.H.S. grant NS-00884 from NINCDS.)

A small but consistent increase in the amplitude and duration of ganglionic afterhyperpolarization. Moreover, GABA antagonists block the electrogenic Na-K pump (see Libet et al., Life Sci. 20: 1863-1870, 1977). PT-HP recorded at non-synaptic ganglia yields primarily a depolarization. Moreover, GABA antagonists block the electrogenic Na-K pump, further supporting the possibility that GABA is the neurotransmitter involved in it. We found also that the dendritic depolarizing GABA response could be elicited in the presence of a low Ca/Na(high K) which blocked synaptic transmission, implying that these iotophoretic responses were not indirectly produced. These results suggested the depolarizing aspect of the electrically stimulated response was mediated by dendritic receptors. We tested this hypothesis with localized pressure injections of TTX (10^-7 M) and found TTX could selectively abolish either hyper- or depolarizing of the electrically evoked response depending on whether it was applied to the soma or dendritic regions, selectively. Blockage of the depolarizing phase occurred while was still possible to elicit soma-sized somatic responses to direct current pulses, indicating the TTX injections were localized and supporting our hypothesis of a dendritic origin of the depolarizing phase.

Both negative and positive phases of the stimulated response were associated with a large conduction increase and had inhibitory effects on the potential generation rate which was increased to those found to occur with iotophoretic GABA. The depolarizing phase of the synaptic response was readily produced with orthodromic activity but only rarely and with high stimulus intensities antidromically. This suggests the dendritic receptors may be activated preferentially as part of a feedforward inhibitory network distinct from the classical posttetanic component.

This work was supported by PHS Grant GM-23478, NIH Postdoctoral Fellowship 9 F32 NS05744-02 (B.E.A.) and NCMR 00278 (R.A.N.).

BIPHASIC IPSPS IN RAT HIPPOCAMPAL SLICE. Bradley E. Alger and Roger A. Nicoll. Depts. of Pharm. and Physiol., Sch. of Med., Univ. of Calif., San Francisco, CA 94143.

Iontophoretically applied GABA has been reported to have a biphasic (negative-positive) effect on membrane potential on certain CNS neurons, without any depolarizing effect being associated with the activation of dendritic receptors. It is not known, however, if the biphasic response can be produced synthetically, nor if the postulated biphasic action represents a unique functional characteristic of the postsynaptic NE: the ganglionic hyperpolarization following a train of antidromic, postganglionic spikes to acetylcholine (ACh) is insensitive to phenoxybenzamine. However, dndro-dendritic junctions between ganglion cells are not explained by the synaptic mediators. (Supported by U.S.P.H.S. grant NS-00884 from NINCDS.)

Elsewhere in this meeting we report that iontophoretic application of GABA to the somatic region of a pyramidal neuron of the hippocampus produced a chloride and picrotoxin-sensitive biphasic response; a hyperpolarization followed by a depolarization. We now describe a similar biphasic response which can be elicited by synaptic rather than iontophoretic release of the inhibitory neurotransmitter if pentobarbital is present in the bathing solution. The response of CAI pyramidal neuron to orthodromic stimulation was recorded by intracellular microelectrodes in hippocampal slices maintained at 34-35°C and bathed in CSF with a potassium concentration of 5 mM. Slices were incubated in 125 mM sodium pentobarbital for at least one hour before the experiment began. Under these conditions, low intensity stimulation elicited a hyperpolarization which lasted from one to two seconds, and which was associated with a marked increase in membrane conductance. Increased stimulus intensities usually evoked a depolarizing component which, onset following the early hyperpolarizing peak of IPSP. A marked increase and prolongation of the membrane conductance also occurred. Topical application of a low chloride solution increased the size of the depolarizing component while shifting its reversal potential to a less negative value. Application of GABA antagonists picrotoxin and bicuculline methiodide reduced the depolarizing component more readily than the hyperpolarizing phase. Repetitive stimulation at a rate of 1 Hz produced a summation of the depolarizing components to a plateau which could also be reduced by picrotoxin or by bicuculline. Although most responses were recorded with potassium-acetate and potassium-sulphate electrodes, similar biphasic responses were also recorded with K-acetate and K-sulphate electrodes. Supported by Grant #NS14443 and NS11753

APOMORPHINE-INDUCED PHOSPHORYLATION OF A SPECIFIC SYNAPTIC MEMBRANE PROTEIN IN STRIATAL SLICES. J. G. D. Bannister and G. Lynch Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

The phosphorylation of synaptic membrane proteins has been postulated to mediate the effects of dopamine in the striatum via the stimulation of a dopamine-sensitive adenylate cyclase. However, until now, there have been no reports that dopamine or its agonists can produce a change in the phosphorylation of specific brain proteins, either in vivo or in vitro. We now report that, in striatal slices, a specific dopamine agonist, changes the phosphorylated state of a specific synaptic plasma membrane (SPM) protein. Further, this protein appears to be the same as that which previously was reported to undergo phosphorylation after induction of long-term potentiation in the hippocampal slice preparation. Striatal slices were incubated with apomorphine (100 µM) for 2 minutes, and either crude mitochondrial or synaptic plasma membranes were prepared. These fractions were then assayed for endogenous phosphorylation, and the samples were then run on an exponential polyacrylamide gel system. Apomorphine induced a marked and specific decrease in the incorporation of 32P into a specific protein with a molecular weight of about 40,000 daltons (40K) in both the crude mitochondrial and the SPM fractions. This effect of apomorphine was totally reversed by incubating the striatal slices with the dopamine antagonist haloperidol (100 µM). In addition, phosphorylase B kinase (P BK), which has been shown to specifically phosphorylate the 40K protein in hippocampal SPMs, was also found to phosphorylate the 40K protein in striatal SPMs. However, this effect was smaller in SPMs prepared from apomorphine-treated slices than in SPMs from control slices. These findings led to the following conclusions:

1. Apomorphine, a specific dopamine agonist, increases the endogenous phosphorylation of a specific SPM protein in the striatal slices.
2. The change in the phosphorylated state of the 40K protein induced by apomorphine in striatal slices and by electrical stimulation in hippocampal slices may be mediated by phosphorylase B kinase.
3. Since dopamine agonists are known to induce long-term behavioral changes, since receptor activation in the hippocampal slice induces long-term potentiation, the initial change in the 40K protein phosphorylation may well represent a consequence of behaviorally enduring changes in neuronal transmission are initiated.

We have investigated the problem of how a non-impulsive neuron can release aminated material with repeated activity in the form of integral numbers of multilamellar packets or quanta. In the peripheral nervous system, quanta can be liberated spontaneously (without any prior event). We have investigated the possibility of a single quanta to the random occurrence of brief depolarizing events termed spontaneous miniature synaptic potentials. So far there has been no demonstration of spontaneous miniature potentials in the mammalian brain. We report that miniature potentials can be recorded in guinea pig hippocampal nerve cells under conditions in which repetitive activity (in the absence of an impulse-dependent) transmitter release have been blocked by TTX (0.5 μM) and manganese (2 μM). This phenomenon was observed regularly in neurons of the CA3 region in the guinea pig hippocampus. The miniature potentials (impulse-dependent) were amplification in example at right: 5 mV and 50 msec.

The results of these miniature potentials were analyzed in 822 neurons in which the frequency of miniature potentials was 5 spikes/sec. In 50% of cases the frequency of miniature potentials was positively skewed, with the smallest events usually merging into the background noise. The mean amplitude of those events emerging above the background noise was 2.1 ± 0.3 μV. The large size of these events was due to the action of the voltage-dependent calcium current which is also present in other neurons. The properties of these miniature potentials are similar to those observed in other neurons, including their sensitivity to voltage-clamp and their sensitivity to voltage-sensitivity. In addition, the miniature potentials are also sensitive to TTX or replacement of sodium with potassium.


The quantum hypothesis of synaptic transmission holds that neurotransmitter is released at some time during the upstroke of the excitatory postsynaptic potential, with the amount of transmitter released proportional to the amplitude of the potential. In the peripheral nervous system, quanta can be liberated spontaneously (without any prior event). We have investigated the possibility of a single quantum to the random occurrence of brief depolarizing events termed spontaneous miniature synaptic potentials. So far there has been no demonstration of spontaneous miniature potentials in the mammalian brain. We report that miniature potentials can be recorded in guinea pig hippocampal nerve cells under conditions in which repetitive activity (in the absence of an impulse-dependent) transmitter release have been blocked by TTX (0.5 μM) and manganese (2 μM). This phenomenon was observed regularly in neurons of the CA3 region in the guinea pig hippocampus. The miniature potentials (impulse-dependent) were amplification in example at right: 5 mV and 50 msec.

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vesicle antigen in crude homogenates of electric organ. Although vesicles, the serum was adsorbed with a membrane fraction from and Biophysics, N.Y.Univ.Med.Ctr.,New York, N.Y. 10016. cells with low distortion from membrane capacitance,whereas vesicle serum binds to nerve terminals of the frog, rat and dies to cholinergic synaptic vesicles purified from the electric NS09878 (to RBK) and a Postdoctoral fellowship from the Muscular IMPULSIVE NEURON. A.R.Blight and R.Llinás .  Dept.Physiology synaptic vesicles have specific antigens, absent from other membranes. This is supported if the neuron is not-impulsive & the form of integral numbers of multilamellar packets or quanta. In the peripheral nervous system, quanta can be liberated spontaneously (without any prior event). We have investigated the possibility of a single quantum to the random occurrence of brief depolarizing events termed spontaneous miniature synaptic potentials. So far there has been no demonstration of spontaneous miniature potentials in the mammalian brain. We report that miniature potentials can be recorded in guinea pig hippocampal nerve cells under conditions in which repetitive activity (in the absence of an impulse-dependent) transmitter release have been blocked by TTX (0.5 μM) and manganese (2 μM). This phenomenon was observed regularly in neurons of the CA3 region in the guinea pig hippocampus. The miniature potentials (impulse-dependent) were amplification in example at right: 5 mV and 50 msec.

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The molecular constituents of synaptic vesicles may be unique or they may be shared by other cellular structures. We have examined this question immunologically by raising rabbit antibodies to cholinergic synaptic vesicles purified from the electric organ of Narcine brevis. We have shown that a significant percentage of the synaptic vesicle antigen exists. This conclusion is supported by the fact that the vesicle serum binds to nerve terminals of the frog, rat dies to cholinergic synaptic vesicles purified from the electric NS09878 (to RBK) and a Postdoctoral fellowship from the Muscular IMPULSIVE NEURON. A.R.Blight and R.Llinás .  Dept.Physiology synaptic vesicles have specific antigens, absent from other membranes. This is supported if the neuron is not-impulsive & the form of integral numbers of multilamellar packets or quanta. In the peripheral nervous system, quanta can be liberated spontaneously (without any prior event). We have investigated the possibility of a single quantum to the random occurrence of brief depolarizing events termed spontaneous miniature synaptic potentials. So far there has been no demonstration of spontaneous miniature potentials in the mammalian brain. We report that miniature potentials can be recorded in guinea pig hippocampal nerve cells under conditions in which repetitive activity (in the absence of an impulse-dependent) transmitter release have been blocked by TTX (0.5 μM) and manganese (2 μM). This phenomenon was observed regularly in neurons of the CA3 region in the guinea pig hippocampus. The miniature potentials (impulse-dependent) were amplification in example at right: 5 mV and 50 msec.

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SYNAPTIC TRANSMISSION

2486 FREQUENCY DEPENDENCE OF GANGLIONIC BLOCKADE BY d-AMPHETAMINE.
Daryl Christ* (SPON: D.L. Avery). Dept. of Pharmacology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72201.

The magnitude of ganglionic blockade by d-amphetamine is dependent on the frequency of preganglionic stimulation (Bowring, Br. J. Pharmac. 44: 71, 1972). The frequency-dependence of ganglionic blockade was explored in the isolated stellate ganglion of the hamster using extracellular recording techniques.

d-Amphetamine reduced the post-ganglionic potential by 86% at 0.2 Hz, and 41% at 2 Hz. The frequency-dependent blockade by d-amphetamine was not affected by propranolol (10-5 M), which was slightly reduced by phentolamine (10-7 M), and was considerably reduced by atropine (10-6 M), suggesting that a muscarinic mechanism is involved.

d-Amphetamine produced spontaneous discharges in the post-ganglionic nerve at 10-3 and 10-4 M. This is indicative of a depolarizing action, and this depolarizing action may potentiate muscarinic transmission in the ganglion. d-Amphetamine reduced the post-ganglionic potential by 86% at 0.2 Hz, and 41% at 2 Hz. The frequency-dependent blockade by d-amphetamine was not affected by propranolol (10-5 M), which was slightly reduced by phentolamine (10-7 M), and was considerably reduced by atropine (10-6 M), suggesting that a muscarinic mechanism is involved.


Catecholamines (CA) have long been known to have potent inhibitory effects on sympathetic transmission. It now appears that a receptor-mediated inhibition of sympathetic ganglionic transmission has been proposed as the primary neurotransmitter of this sympathetic ganglionic response by comparing the relative potencies of three CA's (epinephrine (EPI), norepinephrine (NE) and dopamine (DA)) in producing a hyperpolarizing response at the RSCG.

To examine this post-synaptic effect of CA on ganglionic transmission a concentration-response analysis was performed using an extracellular recording technique in the sucrose-exp. EP1, NE and DA in varying concentrations were administered by superfusing the ganglion.


A recent paper from this laboratory (Collatos et al, Brain Res. 160: 514, 1979) has demonstrated that EPSPs produced in a given motoneuron by a given a fiber exhibit fluctuating synaptic delays. The purpose of this communication is to explore the relationship between the statistical properties of the fluctuations and the EPSPs in cats. We studied relatively large EPSPs (171-1129 µV) with brief rise times (0.3-0.6 msec, 10-90%). We have found that the variance of the latency distribution may be best described by a log-normal distribution.

The problem in the interpretation of these results is that large EPSPs might be easier to pick out of synaptic noise in single sweeps. This would be expected to reduce the variance of the onset latency distribution for large EPSPs artificially. However, if this were so, it would be expected that the distributions would be skewed to the right (long latencies) since we have already observed that the population of EPSPs with short latencies at a given synapse is larger in amplitude on the average than the population with long latencies (as at the neuromuscular junction - Barrett and Stevens, J. Physiol. 227:665, 1972). In fact, about one-half of the distributions were skewed, but in the opposite direction, i.e. in the direction of the larger EPSPs with shorter onset.

This suggests that the variance of EPSP delays is not significantly influenced by difficulties in detection of their onsets at least for this size of large EPSPs. Furthermore, since the distributions which were skewed were restricted almost entirely to synapses at which relatively large EPSPs were generated, it is unlikely that skewness was a consequence of an unfavourable signal to noise ratio. As expected from the above findings, skewed distributions tended to exhibit the smallest variance, the largest variance was not associated with skewness. We conclude that at synapses at which large EPSPs are produced, the average time variability in the release process is less than at synapses where EPSPs are larger in amplitude. The alternative hypothesis, that the fluctuations are the result of increased variability in conduction time through the presynaptic terminal, seems unlikely since (a) the small potential changes recorded from inside the motoneuron and identified as a terminal potential in the presynaptic a fiber (Munson and Supert, Hypothermococse Abst. 78, 1978) exhibits no latency fluctuations despite variability in onset of the subsequent synaptic potentials. It remains to be seen whether these fluctuations can be accounted for by synaptic inhibition at this synapse. (Supported by NH).
A large body of evidence supports the hypothesis that the release of vesicle-contained transmitter occurs via fusion with the presynaptic membrane in the presence of Ca++. It is necessary to know whether or not it is present in higher concentration on the protoplasmic surface of the vesicle membrane. This effect of physostigmine was consistent with the requirement from model studies that the receptor is an ionophore which transports an ion of 100 mV to the outside of the synapse. When applied to a phrenic nerve-diaphragm preparation from the rat, the material in both peaks causes a biphasic release of meps similar to that caused by unfractionated hemolymph. The second phase of release caused by the material in Peak B, however, is more prolonged than the first phase of release caused by the material in Peak A. Further purification and characterization of the active components of Peaks A and B should provide useful tools with which to elucidate the mechanism of release of acetylcholine.

Supported by the National Science Foundation (BNS 76-80657) and the Nelson Research and Development Co.

The slow postsynaptic potentials of the superior cervical ganglia (SCG) of the rabbit, rat and guinea pig were studied by means of the sucrase-gap technique. In the presence of hexamethonium (C6, 0.5 mM) which suppressed the initiation of ganglion compound action potentials, repetitive preganglionic stimulation increased the amplitude of P potentials in guinea pig SCG superfused continuously with C6, repetitive preganglionic stimulation ended the appearance of LN after 15-20 min drug treatment. The LN was replaced by a hyperpolarizing potential. This effect of hexamethonium was fully reversible. The effects on P and LN potentials of a number of adrenergic and dopaminergic antagonists were studied. Alpha-adrenergic blockers, phentolamine and clonidine induced depressions in P potentials. The decline in LN was much larger than LN in all three species. These results demonstrate that muscarinic receptor is involved in the generation of P and LN potentials in mammalian sympathetic ganglia, whereas the cholinergic nature of P is less certain. (Supported by NS 06455 and American Parkinson Disease Foundation).

All three major classes of retinal ganglion cells terminating in the goldfish optic tectum appear to be nicotinic-cholinergic (Schmidt and Freeman, Brain Res., in press, 1979). In order to examine the localization and density of nicotinic acetylcholine receptors (nACHR) on physiologically tissue slices, 100-300 µm thick were made parasagittally through the tectum, by cooling the fish to 40°C, rapidly removing the tectum and placing it on a Peltier-effect cooled movable stage, using a metacrylate adhesive. Slices, made by rapid vertical strokes of a razor blade, were then transferred to a coverslip which formed the bottom of a perfusion chamber, and were viewed with an inverted microscope equipped with Nomarski optics and a Zeiss fluorescence epi-illuminator. Individual cell bodies and their axonal and dendritic processes could be clearly seen (mag. 500X), as could the 3 layers of retinal cell bodies and their axonal and dendritic processes could be clearly seen (mag. 500X), as could the 3 layers of retinal fiber layers, and stable intracellular resting potentials (25-65 mV) were recorded from cell bodies in the superficial and deep gray layers (diam. 7-12 µm, Zn 70-120 µM). The specific aim of this study was to provide an integrated approach to the measurement of intactness of synaptosomes during isolation and incubation using LDH activity and electron microscopy. Synaptosomes isolated on unbuffered sucrose gradients were compared with those isolated on sucrose gradients of known pH. After several different solutions of synaptosomes were centrifuged and both supernatant and pellet were assayed for LDH activity. In addition, effects of the incubation on synaptosomal morphology were also determined. By using this approach, we have outlined a basic technique for the optimum use of synaptosomes as a model system for the investigation of presynaptic events.
SIMPON TRANSMISSION


Synaptic transmission within the superior cervical ganglion (SCG) has been extensively studied because it is experimentally accessible and contains a diversity of types of synaptic transmission. Further experimental simplification has been achieved using tissue culture techniques, so that the bioelectrical basis of neural transmission and its correlation with electrophysiology have been usefully studied. It is puzzling, therefore, that the slow synaptic potentials seen in intact animals and isolated whole organ preparations have not been discovered in primary cultures of mammalian SCG (O’Lague, P. et al., Devel. Biol. 67: 424, 1978).

We have consistently found slow depolarizing potentials in response to iontophoresis of acetylcholine (ACH) onto neurons dissociated from neonatal rat SCG. With small pulses of ACh a fast depolarizing response associated with a fall in membrane resistance (Rm) is evoked. When the fast response reaches saturation with large pulses of ACh, a long-lasting depolarization of delayed onset becomes evident. The slow ACh response increases in amplitude and duration in a dose dependent fashion, a maximal response lasting 30 to 49 sec. The Rm during the slow response either remains unchanged or increases slightly. Cursore eliminates the fast ACh potential without affecting the slow potential. Excitability is increased during the muscarinic action of ACh. This effect is independent of ACh induced changes in membrane potential. In some cells repetitive hyperpolarizing the membrane with steady inward current reduces the amplitude of the ACh depolarization but does not reduce the increased excitability effected by ACh. The spike afterpotential is reduced following muscarinic ACh activation, and this effect, too, occurs separately from changes in membrane excitability.

The requirement of a large ACh dose to evoke the slow depolarizing potential may suggest that both nicotinic and muscarinic ACh receptors are involved. The amine component of the gap junction (Heilbronn, E. and T. Bartatzi, Prog. in Neurobiol. 11: 171, 1978) must be saturated before lower affinity muscarinic receptors mediating the slow response can be evoked. Our findings offer the opportunity to more easily and unambiguously study the ionic and biochemical mechanisms of muscarinic ACh action.


The sucrose gap recording technique was used to study post-train hyperpolarizing potentials in the cat parasympathetic vesical pelvic ganglia (VPG). This is the first time this technique has been employed to record both nicotinic and muscarinic slow membrane potential changes from a parasympathetic ganglion.

Following nicotinic blockade of the ganglion with either hexamethonium (10--8M), curare (10--7M), and morphine (500 µg/ml), and orthostatic stimulation of 30 Hs for 1 sec, two types of postsynaptic potentials were observed. First, a slow inhibitory postsynaptic potential (slow ipsp) was observed, with an amplitude of 0.5-1.2 mV and duration of 5-9 sec. Second, a long lasting hyperpolarization that was small in amplitude (0.1 to 0.4 mV) but lasted up to 3 min. The long lasting hyperpolarization was more apparent after blockade of the slow i.p.s.p. No slow or late excitatory postsynaptic potentials were observed (825).

The slow i.p.s.p. could be blocked by atropine (10--7-10--6M) but was not blocked by phentolamine (10--7-10--6M) or propranolol (10--7M). On the other hand, NE (10--8M) added to the bath by superfusion depolarized the membrane; this depolarization was blocked by phentolamine (10--7M). The long lasting hyperpolarization was not blocked by atropine, phentolamine or propranolol.

In conclusion, the long lasting hyperpolarization persisted in the presence of zero Na+. The slow long lasting hyperpolarization was probably not due to synaptic events, but rather resulted from the repetitive volley being conducted in axons or fibers traveling through the ganglion. The amplitude and duration of the long lasting hyperpolarization could be enhanced by increasing the frequency and duration of train stimulation.

These data suggest the presence of an inhibitory postsynaptic potential that is mediated via a muscarinic receptor. Hartnell et al. (1977) have found a similar muscarinic i.p.s.p. in parasympathetic ganglion cells that modulate heart beat of the amphibian mudpuppy.


PURIFICATION OF AN α-BUNGAROTOXIN BINDING COMPONENT FROM DRUOOPHILA MELANOCASTER. Janice I. Cepner and Linda M. Hall.

An α-bungarotoxin binding component was solubilized from homogenates of Drosophila heads as an initial step in the purification of this component. Determinations of hydrophile-lipophile balance (HLB) numbers of 13 to 14.5 were the most effective in solubilizing toxin binding activity. An HLB number reflecting a relative hydrophilicity of a detergent) Optimal solubilization was achieved with 1X Triton X-100 in 1.0 M NaCl. Under these conditions, a maximum of 50% of the activity was solubilized.

The solubilized α-bungarotoxin binding component was purified by passage twice through an affinity column composed of Sepharose 4B covalently linked to the α-bungarotoxin from the cobra Naja naja elasmoides. The binding component which was adsorbed to the affinity column and eluted with carbamylcholine had a molecular weight of approximately 500,000 by agarose gel chromatography and 250,000 by sucrose gradient analysis. The specific activity of the final purified α-bungarotoxin binding component was 1 umole α-bungarotoxin binding sites/g protein, representing a 700 to 1000-fold increase over that of the solubilized extract.

Cholinergic compounds were shown to inhibit [125I]α-bungarotoxin binding to the purified extract. The concentrations required for 50% inhibition are as follows: dihydro-β-erythroidine (0.026 µM), nicotine (0.25 µM), d-tubocurarine (0.66 µM), triethanolamine (1.3 µM), gallamine (1.6 µM) acetylcholine (3.0 µM), atropine (18 µM), carbamylcholine (23 µM), decamethonium (63 µM) and cromolyn (150 µM). A specific activity of 6000 CPM/µg of protein was assayed.

The characteristics and properties of the affinity column which was bound to the affinity column and eluted with carbamylcholine are similar to that of the purified toxin binding to the purified extract. The concentrations required for 50% inhibition are as follows: dihydro-β-erythroidine (0.026 µM), nicotine (0.25 µM), d-tubocurarine (0.66 µM), triethanolamine (1.3 µM), gallamine (1.6 µM) acetylcholine (3.0 µM), atropine (18 µM), carbamylcholine (23 µM), decamethonium (63 µM) and cromolyn (150 µM). A specific activity of 6000 CPM/µg of protein was assayed.

We have examined in detail synaptic depression, frequency facilitation and post-tetanic potentiation (PFP) at the SCG synapse onto 19,91,02 (tentatively identified) for comparison with RCI-15. Identical results were obtained by stimulating RCI-15. The short-term plasticities (synaptic depression and frequency facilitation) are quantitatively similar at the two synapses. The rising phase of PTP is, however, slower at RCI-15 (t<.001, n=19) than at RCI-15. We have evidence to suggest that these differences in PTP parameters are due to functional differences in the pre-synaptic terminals and are not due to heterosynaptic influences which can accelerate PTP decay.

Supported by the Veterans Administration and by an NSF Graduate Fellowship (PFC).
2500 PRE AND POST SYNAPTIC EFFECTS OF ELEVATED EXTRACELLULAR POTASSIUM UPON IN VITRO HIPPOCAMPAL SLICES. John J. Habits and Arvid Lundelevold. Institute of Neurophysiology, University of Oslo, Oslo, Norway.

Accumulation of potassium in the extracellular space following repetitive neural activity has been repeatedly documented in the mammalian central nervous system. Subsequent changes in neuronal responsiveness during these periods have been observed. Although these changes in extracellular potassium (K+) have been shown to have particular spatial and temporal characteristics, it has not been possible to clearly delineate pre and post synaptic effects. The present study investigated the effect of manipulation of the ionic microenvironment upon extra-cellularly recorded indexes of pre and post synaptic activity in the in vitro hippocampal slice.

Transverse slices of guinea pig hippocampus, 400-500 microns thick, were maintained in vitro. Slices were initially incubated in a medium containing 3.25 mM (K+) and KCl was subsequently increased to 6.25-12.25 mM and again all activity was abolished in 15.25 mM. Low impedance glass micropipette recording electrodes were placed in stratum radiatum and the cell body layer of CA1 to record the pre synaptic fibre volley (PV), extracellular EPSP and population spike (PS) resulting from stimulation of the Schaffer collaterals. For a given constant input strength as measured by stimulus current amplitude and of PV, the population spike amplitude was directly related to the range 3.25-12.25 mM. At high stimulus strengths multiple PS's were seen at (K+) concentrations above 12.25 mM and occasionally in 12.25 mM. These effects on firing and size of the PS were reversible. Elevation of (K+) also increased the size of the EPSP in response to a constant input strength in the range of (K+) concentrations 3.25-12.25 mM. At 15.25 mM an occasional subsequent degression of EPSP amplitude was observed in 15.25 mM and again all activity was abolished in 15.25 mM.

These results suggest that raising (K+) increases excitability by effecting changes at both pre and post synaptic sites. Post synaptic changes can be attributed to a depolarizing action of (K+), while the pre synaptic EPSP changes may result from a specific effect of (K+) on the transmitter release system as described for the neuromuscular junction (Cooke & Quastel, 1973).

2501 AMINO ACIDS THAT EXCITE CEREBELLAR PURKINJE CELLS AND THEIR POTENTIAL ROLE AS CLIMBING FIBER AND PARALLEL FIBER NEUROTRANSMITTERS. J.J. Hackett, J.L. Cochran, and D.J. Brown. Dept. of Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908

The climbing fiber-evoked, Purkinje cell (PC) EPSP is unitary, and its quite large amplitude easily distinguishes it from graded EPSPs evoked by parallel fiber stimulation. We have suggested that the glutamatic (Glu) may be the releasable transmitter from parallel fibers but not from climbing fibers, since the reversal potential for Glu-evoked postsynaptic potentials is significantly different from the reversal potential for Ca2+ (Glu). We have examined this notion in an investigation of the Glu receptors with putative antagonists and with L-aspartic acid (Asp).

Glia were damaged by iontophoresis from turtles. Saccular sections (200-300 µm thick) of cerebellum were superfused with a Ringer solution from a system that permitted rapid exchange of extracellular solutions. Iontophoretic responses of known types of agents. PC responses were recorded extracellularly and intracellularly with microelectrodes filled with 2 M K-citrate. We have found no distinguishing characteristics between the actions of Glu and Asp on PC responses. Thus both agents depolarize PC to the same extent, with the same time course, and over the same concentration range (2 x 10^-6 to 5 x 10^-5 M). Several putative antagonists of Glu, Asp and several of the agents suggest that they may be acting at the same receptor site. Based on a comparison of reversal potentials, the PC site of action is more likely the subsynaptic receptors for the parallel fiber neurotransmitters than those for the climbing fiber. However, high concentrations of morphine may have acted to block some postsynaptic effects of Glu, Asp and several putative antagonists. Support: SK2O DA 00009 from NIDA and BBS 77-155271 from NSF.

2502 VOLTAGE DEPENDENCE OF SYNAPTIC CURRENTS AT A CNS SYNAPSE IN THE MANTIS. W.M. Hopper, John W. Dyke, and Michael J.L. Bennett. Dept. Neurosci., Albert Einstein College of Medicine, Bronx, NY 10461

The Mauthner fibers form axo-axonic chemical synapses on giant fibers just beneath the floor of the Ith ventricle. Two to six giant fibers on each side arise from their cell bodies in the lateral wall of the Ith ventricle, pass medially dorsal to the near Mauthner fiber from which they receive a single synapse and then bifurcate to form anteriorly and posteriorly running branches that reach several synapses in the far Ith ventricle (Model et al., Brain Res., 45:288, 1972). The transmitter appears to be ACh (Spira et al., J. Cell Biol., 47:199a, 1970). The two Mauthner fiber PS's in the giant fibers can be individually evoked by graded stimuli applied to the caudal spinal cord. Iontophoresis of the dye, Fast Green, into a giant fiber had no profound effect. If current levels were increased by current decay time or amplitude, and facilitated simultaneous penetration with current and voltage electrodes. Electrodes were positioned within 50µ of one another and within 100µ of the synapse from the near Mauthner fiber. The post synaptic region was then voltage clamped at its resting potential and stepped to various levels 15 msec before antidromic activation of the presynaptic cells. At the resting potential (-85 µV) the post synaptic currents rise to a peak in less than 0.1 msec; after an initial more rapid phase, decay is exponential with a time constant of c.0.60 msec. Hyperpolarization increases the peak current and slows the decline of (-0.85 µm at -160 µV). Increasing inside positivity reduces and then inverts the synaptic currents. The peak amplitudes are essentially abolished by reversal potentials of -10 µm. Reduced and inverted currents decline more rapidly (c.0.26 msec at 90 µV). Peak amplitude is linearly related to voltage. These results are consistent with data from the frog neuromuscular junction (Magleby and Stevens, J. Physiol., 223:173, 1972) in which the decline in synaptic current is more rapid at more negative potentials. The sensitivity of the Mauthner synapse is determined by the rate of closing of ACh activated channels. This investigation was supported by NIH training grant number 5T32GM7288 from NIGMS.


Primary afferent depolarization in the spinal cord and brain stem is believed to be causally linked to presynaptic inhibition (Schmidt, R.P., Ergeb. Physiol. 63:20, 1971). Using the isolated olfactory bulb of the bullfrog, we have recorded by the sucrose gap method a similar, but much longer lasting, depolarization of the olfactory primary afferents following stimulation of the same or adjacent olfactory nerve (ON) fibers. The ON depolarization lasted up to 40 sec, increasing in amplitude and duration with increasing stimulus strength. These results indicate that intact synaptic transmission is necessary for the ON depolarization but that firing of the ON afferents is not a prerequisite. The antidromic compound action potential evoked by stimulation of the primary afferent terminal area was blocked by 2 µM tetrodotoxin (TTX). TTX blocked the ON depolarization. However, L-glutamic acid (Glu) at 5 x 10^-4 M was added to the superfusing solution and it was found that the ON depolarization lasted up to 40 sec. TTX blocked the ON depolarization. The ON depolarization was prolonged by Ca2+ (Glu) but not by L-aspartic acid (Asp). Glu. Although some of these agents may have acted to depolarize presynaptic nerve terminals, Glu-evoked depolarizations were not reduced by a Ringer solution containing lowered Ca2+ and Mg2+ which effectively blocked all synaptic transmission. In conclusion, the similar depolarizing effects of Glu, Asp and several putative antagonists suggest that they may be acting at the same receptor site. Based on a comparison of reversal potentials, the Glu site of action is more likely the subsynaptic receptors for the parallel fiber neurotransmitters than those for the climbing fiber. However, high concentrations of morphine may have acted to block some postsynaptic effects of Glu, Asp and several putative antagonists. Support: SK2O DA 00009 from NIDA and BBS 77-155271 from NSF.
2506 ROLE OF CATECHOL-O-METHYLTRANSFERASE (COMT) IN SLOW POSTSYNAPTIC RESPONSES TO PREGANGLIONIC IMPULSES, IN MAMMALIAN SYMPATHETIC GANGLION. B. Libet and John H. Ashe, Department of Physiology, School of Medicine, University of California, San Francisco, CA. 94143.

The COMT-Inhibitor U-0521 (0.3µM) produces a reversible augmentation of both the dopamine (DA)-mediated s-EPSP and the ACh-mediated s-EPSP postsynaptic responses to preganglionic nerve stimulation, in rabbit superior cervical ganglia. The relative increases in both slow PSP's were larger for the weaker test responses (to a single volley), but the overall potentiations were greater and more consistently apparent for the s-EPSP.

Augmentation of s-EPSP is explained as due to potentiating of the long-lasting DA-modulatory enhancement of the s-EPSP response to ACh (acting muscarinically) (Libet and Tosaka, Proc. Natl. Acad. Sci. 62: 667-673, 1970). This implies that DA spontaneously released by the dopaminergic interneurones (“SIF” cells) can be sufficient to induce a modulatory change, when this DA is protected from inactivation by COMT. This view is further supported by finding that bromocriptine (7µM), an antagonist of DA-stimulated adenyl cyclase in brain, seems to increase during ontogenesis.

The present evidence also suggests that COMT can play a significant limiting role that is analogous to that of acetylcholinesterase, in at least some neurones where catecholamine functions involving catecholamine transmitters. For DA in this ganglion, COMT-access barriers to limit the DA-modulatory action, if present, in some of the experiments is acknowledged.

Ruthenium Red, seems to increase during ontogenesis.

(Grant NS-00884 from NINCDS.)
SYNAPTIC TRANSMISSION


We have continued an intracellular analysis of synaptic potentials in the isolated turtle olfactory bulb preparation (Nowicky, Waldow and Shepherd, Neurosci. Absts. 4:1583, 1978). Single olfactory nerve volleyes induce a complex sequence of synaptic potentials in mitral cells. There is an early period which includes the EPSPs in the terminal tufts of mitral cell dendrites in the olfactory glomeruli, and a later period consisting of a slow hyperpolarizing IPSP. The IPSP can be divided into two phases, early and late. The early phase covers the first several hundred msec of the response, whereas the later phase lasts for very long periods; in some cases the hyperpolarization persists as long as 4 secs following a single volley. When the membrane potential was held at different levels by injected current, the early IPSP was found to have a reversible potential of ~70 to ~80 mV. In this respect it resembled IPSPs in motoneurons and other central neurons. Some degree of overlap with the initial EPSP may be present during this early phase. In contrast, the late IPSP did not reverse; it decreased in amplitude as the membrane was depolarized, and also when it was hyperpolarized. This is similar to the behavior of voltage-sensitive IPSPs previously demonstrated in piriform cortex (Mori, Math. Proc. Jap. Acad. 54:484, 1978) and in parasympathetic ganglion cells (Harrzelli, Kuffler, Stickgold and Yoshikami, J. Physiol. 271:817, 1978). Tests with a wide range of intracellular fillers indicated that membrane conductance was increased during the early phase. The results suggest at least two different types of synaptic receptors or receptor mechanisms for inhibition of mitral cells.


The neurohormones vasopressin and oxytocin are synthesized and packaged as pro-hormones in cell bodies in the hypothalamus and transported down the axons to the axon terminals in the posterior part of the pituitary, where the proteins are released into the bloodstream as the neurohormones.

Intracellular injection of the marker horseradish peroxidase (HRP) reveals that ventral and lateral T-cells do not make direct contact with the S-cell; therefore, another neuron must mediate the electrical connection between them. Intracellular injection of the S-cell with fluorescent dye of low molecular weight, such as Lucifer Yellow (Stewart, Cell 14: 741, 1978) or 6-carboxyfluorescein, which can cross between electrically coupled cells (Strehlow et al., Biophys. Soc. Absts. 4:583, 1978), allowed visualization of the synaptic connections. Mitral cells were selectively eliminated from the ganglion with intracellular injection of promastigote axonal "swellings" and glia form the neurohypophysis, from which both hormones are released by exocytosis.

A complete account of this work is in press.[5].


2510 DIRECT ELECTRICAL SYNAPTIC CONNECTION IS MEDIATED BY AN INTERNEURON. Kenneth J. Muller and Sheryl A. Scott. Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Md. 21210.

Touch sensory neurons (T-cells) in segmental ganglia of the leech, stains with nearly uniform intensity the S-cell and a portion of the T-cell's presynaptic terminals. This branch-point failure occurs in response to natural stimuli, and by modulating synaptic transmission can affect integration within the ganglion.

Intracellular injection of the marker horseradish peroxidase (HRP) reveals that ventral and lateral T-cells do not make direct contact with the S-cell; therefore, another neuron must mediate the electrical connection between them. Intracellular injection of the S-cell with fluorescent dye of low molecular weight, such as Lucifer Yellow (Stewart, Cell 14: 741, 1978) or 6-carboxyfluorescein, which can cross between electrically coupled cells (Strehlow et al., Biophys. Soc. Absts. 4:583, 1978), allowed visualization of the synaptic connections. Mitral cells were selectively eliminated from the ganglion with intracellular injection of promastigote axonal "swellings" and glia form the neurohypophysis, from which both hormones are released by exocytosis.

A complete account of this work is in press.[5].


2511 RELEASE OF 8-ADRENERGIC AGONIST FROM SYMPATHETIC NEURONS IN CO-CULTURE WITH PINAL CELLS. Andrew Parfitt* and William Blom (Brain Res. Inst., Neurochemistry, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Co-cultures of pineal cells and sympathetic neurons were prepared from dissociated pineal and superior cervical ganglia dissected from 2-day-old rats. Routinely 300,000 pineal cells were plated with 30,000 neurons. Cultures were maintained in Ham's F12 containing 10% fetal bovine serum and 0.1% bovine insulin at 37°C. The medium was changed every third day. Experiments were done 5 to 8 days post-culture preparation. Test compounds were incubated with the cultures for 6 hours in Krebs buffer. In order to assess endogenous or cocaine-stimulated culture were incubated with compounds that block the reuptake of norepinephrine by nerve endings. Both desmethylimipramine (DMI) (10-5 M) and cocaine (10-4 M) caused an increase in extracellular (E) and intracellular (I) concentration of norepinephrine. Imipramine (I) was blocked by cocaine (C). These conditions were sufficient to block electrically initiated action potentials in the cultured neurons. The increase in NAT activity following treatment with DMI and cocaine varied between preparations, probably as a result of variations in the numbers of neurons in the cultures prepared on different days. This increase in NAT activity was not less than 50% and frequently approached 80% of the activity elicited by a maximum effective concentration of ISO (10-7 M). In order to evoke release from neurons, co-cultures were incubated with batrachotoxin (BTX, 10-7 M), which is reported to stabilize voltage-dependent sodium channels in the open configuration, thus depolarizing the neurons. BTX treatment elicited an increase in NAT activity, which was blocked by simultaneous application of BTX, but was only slightly diminished by treatment with 4x10-5 M MgCl2. Cobalt was tested but was found to kill both the neurons and the pineal cells. Neither TTX nor BTX had an effect on basal or ISO-stimulated NAT activity in pineal cells. Cultures medium taken from the BTX- or DMI-treated co-cultures was not enough to release NAT to the medium when added to cultures of pineal cells alone. This enabled us to bioassay 8-adrenergic agonist released by the neurons in co-culture. Calculations on data from a representative experiment indicated that an amount of 8-agonist equivalent to 6.7x10-12 moles of norepinephrine was released from each neuron during a 6-hour treatment with BTX.


Miniature end-plate currents (m.e.p.c.) recorded at vertebrate neuromuscular junctions are produced by the release of quanta of acetylcholine (ACh), not all of which give rise to transient high concentrations of transmitter in the synaptic cleft. The m.e.p.c. height will be dependent on the relative likelihood of ACh interacting with receptor rather than breaking down to diffuse or diffuse. The dihydropicrotamine (d-TC) is less effective at reducing m.e.p.c. height after injection of acetylcholinesterase (AChE) and is more effective after partial receptor inactivation by synaptic nicotinoopeptide C or o-bungarotoxin. With a few assumptions, a simple equation can be derived which relates the apparent potency of d-TC under varying conditions. The m.e.p.c. height in the absence of d-TC. This provides a basis whereby one can estimate the m.e.p.c. height that would have been achieved if all of the ACh in a quantum were bound to receptor, and the ratio of m.e.p.c. height to this maximum gives an indication of the fraction of ACh in a quantum that has become bound to receptor at the peak of the m.e.p.c. Although the equation ignores a number of complicating factors, it accurately describes the behavior of a variety of mathematical models of the synapse where the m.e.p.c. heights and decay rates of diaphragm indicate that normally about 75% of ACh released as a quantum binds to receptor prior to being hydrolyzed. Katz and Miledi (J. Physiol. 231, 549, 1973) have pointed out that d-TC increases m.e.p.c. decay rate after inhibition of AChE, established by the fraction of ACh that is not bound to receptor and is free to diffuse from the cleft. This phenomenon can be used to estimate the fraction of ACh bound to receptor during the decay phase. Thus, the m.e.p.c. height is a function of AChE.

The effects of varying Ca++ concentration on synaptic transfer.
USE OF DOPAMINE β-HYDROXYLASE ANTIBODIES IN THE STUDY OF VESICLE TRANSMISSION

Robert A. Rush*, Thomas J. Kiliaris, Laurence B. Geffen*, Centre for Neuroscience, School of Medicine, Flinders University, Bedford Park, S. A. 5042, Australia.

Systemically administered antibodies to dopamine β-hydroxylase are taken up into noradrenergic nerve terminals by binding to membrane bound dopamine β-hydroxylase molecules which become exposed on the external surface of the nerve during exocytosis. In the absence of complement, the antibody leads to a lysis of the plasma membrane, resulting in the degeneration of the whole axon terminal. When lysis is prevented by the use of an Fab fragment of the antibody or in the absence of complement, the binding of antibody to DBH results in the inhibition of enzyme activity. The inhibition of enzyme activity may be useful in the determination of DBH in nerve terminals. With the use of an appropriate label, the bound antibody may also be visualised at both the light and electron microscope level. When DBH is coupled to horseradish peroxidase (HRP) and administered either systemically or locally into the anterior chamber of the eye, the HRP-DBH complex can be localised in membrane structures or within axon terminals. In this way, the fate of vesicles which have participated in the release process can be studied.

Twenty-four hours after systemic injection, the sympathetic nerve terminals of the iris contain densely labelled small vesicles. In addition, label was also clearly visible within large membrane organelles and cylindrical cistermae. Little staining was visible on the plasma membrane of axon terminals suggesting that, following exocytosis, the vesicle membrane is rapidly retrieved. This novel approach to the ultrastructural localisation of DBH within nerve terminals may overcome many of the difficulties associated with in vitro immunohistochemistry and moreover provides a method for studying the fate of synaptic vesicles after they have undergone exocytosis.

RELEASE OF ENDOGENOUS CATECHOLAMINES IN THE CAUDATE NUCLEUS AND PREOPTIC AREA OF THE ANTERIOR HYPOTHALAMUS OF FREELY MOVING RATS


The release of endogenous catecholamines (CA), norepinephrine, dopamine and serotonin, was studied in vivo, using a push-pull perfusion technique followed by high pressure liquid chromatography with electrochemical detection (Bioanalytical Systems). Push-pull cannulae were connected to stainless steel hubs (David Kopf) and tubing (24g for push, 32g for perfusion, Small Parts) and machine screws (Small Parts). Cannulae were implanted stereotactically, under chloral hydrate anesthesia (350mg/kg), in either the caudate nucleus or the preoptic area of the anterior hypothalamus of rats. Both the push and pull ends of the cannulae were connected, via atrumatic tubing (Stil-Med) to the output and input ends respectively of miniature electrically pulsed pumps (Valcor), mounted directly on a mercury commutator, thus allowing the rat free movement during the push-pull perfusion technique followed by high pressure liquid injection of amphetamine (5 mg/kg) at the end of the session. The release process was determined under basal conditions during waking and REM sleep and during the non-rapid eye movement sleep. Preliminary results indicate that when DBH is coupled to horseradish peroxidase (HRP) and administered either systemically or locally into the anterior chamber of the eye, the DBH-DBH complex can be localised in membrane structures or within axon terminals. In this way, the fate of vesicles which have participated in the release process can be studied.

SYNAPTIC VESICLE MORPHOLOGY IN THE RAT NEUROMUSCULAR JUNCTION FOLLOWING APPLICATION OF LEPTOMARTIN

A.A. Sadun, A.K. Cree*, T.R. Heaston* J.R. Stoner*, U.O. McClure. Neuro. Res. Lab., Huntington Institute of Applied Medical Research, 90200, Huntington, Calif. 91034. (USA), 75, 3512, 1978). In the hippocampal slice both theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theophylline, a phosphodiesterase inhibitor, and theo...
were washed free of HA, normal sensitivity to HA rapidly returned was dependent on the concentration of HA used to desensitize (at 50 µM, t1/2 = 5 min; at 100 µM, t1/2 = 2 min). When the cells were washed free of HA, normal sensitivity to HA rapidly returned and the rate of resensitization was independent of the concentration of histamine used to desensitize (half-time ≃ 8 min). Data from the dose-response curves using cells partially desensitized with HA suggested a loss of agonist binding sites. However, binding studies with the H1 receptor antagonist, (3)piperidine, to whole cells showed that there was no significant change in the number of antagonist binding sites associated with short-term desensitization. These data for short-term desensitization fit the following cyclic model as described by Gosselin:

HA + receptor → HA + receptor (active) → cyclic GMP formation

HA + receptor → receptor (inactive)

The nature of the inactive receptor for short-term desensitization is not certain, but could result from a reversible change in receptor conformation which only affects binding and not desensitization. Long-term exposure (> 4 hrs) to HA resulted in a desensitization that was only slowly and partially reversible. Furthermore, this long-term desensitization was accompanied by a marked decrease in the number of specific (3)piperidine binding sites on intact cells. These results suggest that different mechanisms are involved in the short-term and in the long-term desensitization of histamine H1 receptors. (Supported by Natio Foundation and USPHS Grants GM 27692, MH 07295 and DA 1490.)

Supported by Grant FNS14433 and RS11753


Changes of membrane potential during the firing of GABA were recorded from pyramidal neurons in slices of hippocampal cells maintained at 34-35°C and bathed in a synthetic CSF with a K+ concentration of 3.5 mM. As others have reported, iontophoresis of GABA in the pyramidal cell layer results in a superimposition of action potentials when recorded extracellularly. However, intracellular recording revealed that the changes in membrane potential during GABA iontophoresis near the soma may be biphasic: an early hyperpolarization followed by a depolarization. The hyperpolarization had properties similar to those of the IPSP; an increased membrane conductance, the same reversal potential as the IPSP, and sensitivity to picrotoxin. The depolarizing phase was accompanied by an increase in membrane conductance, it was reversed by transmembrane injection of outward currents, and it was abolished by picrotoxin. Topical application of a low Cl- solution reversibly decreased the size of the hyperpolarization and shifted the equilibrium potential of the depolarization. Our results suggest that different mechanisms are involved in the short-term and long-term desensitization of histamine H1 receptors. (Supported by Natio Foundation and USPHS Grants GM 27692, MH 07295 and DA 1490.)

Supported by Grant FNS14433 and RS11753


Methylytransferases can be inhibited by 5'-adenosine homocysteine (AdoHcy) or by analogues of this compound. Analogues which inhibit methylytransferases directly include 3'-deazaadenosine (DZA), adenosine-2', 3'-diado- 5'-carboxamide (744-99), 5'-deoxy-5'-isobutylthioadenosine(SIBA) and 5'-deoxy-5'-isobutylthioadeno- sine (SIBA 5'). Richards and Cantoni, 1977, Mol. Pharmacol. 13:939-947; Chiang et al., 1978, Biochem. Biophys. Res. Commun. 82:417-423.)

Neurons from 8-day chick embryos retained in a culture with rat striated muscle cells for 24 hr formed synapses. Synapses were detected by recording spontaneous depolarization of synaptic membrane potentials with intracellular microelectrodes (Puro and Nirenberg, 1976, Proc. Natl. Acad. Sci., USA, 73:3544-3548; Ruffolo et al., 1978, Proc. Natl. Acad. Sci. USA, 75:2281-2285). These synapses were investigated with the above probes to determine whether methylation reactions occur during synapse formation or neurotransmission. After treating the cultures for 2 hr with one of the AdoHcy or inhibit methylytransferases, had no effect. DZ-SIBA inhibited the muscle responses with a half-time of 3.5 min. No inhibition of the number of synaptic responses was observed. Incubation of the number of muscle responses/min by DZA was reversed within 2 hr after removal of the compound. Further, homocysteine thiolactone potentiated the inhibition by DZA by 6-fold when the two compounds were added together. These results strongly sug- gest that a methylytransferase mediated reaction occurs during neurotransmission.


PC12, a clonal line of rat pheochromocytoma, synthesizes acetylcholine and catecholamines, stores each in different granules, and exhibits a spontaneous and depolarization-induced, Ca2+ sensitive release of each. These properties make PC12 a potential organism to test the hypothesis that the uptake of neurotransmitters to the storage granules is a saturable process.

Methyltransferases can be inhibited by 5'-adenosine homocysteine (AdoHcy) or by analogues of this compound. Analogues which inhibit methylytransferases directly include 3'-deazaadenosine (DZA), adenosine-2', 3'-diado- 5'-carboxamide (744-99), 5'-deoxy-5'-isobutylthioadenosine (SIBA) and 5'-deoxy-5'-isobutylthioadeno- sine (SIBA 5'). Richards and Cantoni, 1977, Mol. Pharmacol. 13:939-947; Chiang et al., 1978, Biochem. Biophys. Res. Commun. 82:417-423.)

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SYNAPTIC TRANSMISSION

2524 STIMULATION-INDUCED RELEASE OF 3H-MORPHINE-PERIUM from EXPLANTS of the RAT SUPERIOR CERVICAL GANGLION: EFFECT OF ALPHA-ADRENERGIC AGENTS. B.D. Walls and H.B. Thoa*. (Spon: H.W. Colburn). Howard Univ., Washington DC 20059 and NIMH, Bethesda, MD 20205. Alpha-adrenergic receptors have been identified in the rat superior cervical ganglion (SCG) (Kafka and Thoa, Biochem. Pharmacol. in press). In culture, SCG cells were found to develop axonal sprouts with physiological and pharmacological properties of adrenergic nerve endings (Sillibourne and Hori, J. Physiol. 244, 1974-1976). A calcium-dependent release of 3H-Morphine (3H-MOR) could be elicited in the cultured SCG with electrical field stimulation (Vu, Thoa and West, The Pharmacologist, 1979). In this study, the effect of alpha-adrenergic agents on release of 3H-MOR elicited by field stimulation (stimulated release) was examined. Rat SCG were cultured for 48 hours and labeled with 0.3nM of 3H-MOR in the continued absence of cationic amino acids. The preparations were then perfused with a modified Krebs solution. After 60 min., electrical field stimulation (3Hz, 20 volts, 5 msec duration) was applied for 1 min., and 4 min. samples were collected prior to and immediately following stimulation. 3H-MOR and its deaminated metabolites 3H-DOMA and 3H-DOPEG were assayed. Perfusion collected in the pre-stimulation period contain mostly 3H-DOPEG (51%) and smaller amounts of 3H-MOR (26%) and 3H-DOMA (23%). Field stimulation of a single electrode (1 sec) to the preganglionic (cervical sympathetic) nerve and to extracellular Ca++. Increasing extracellular Ca++ from a control level (2.2 mM) to 8.0 mM reduced the EF50 for depression to extracellular Ca++. Although not always entirely normal in appearance, diamide-treated terminals typically do contain vesicles, even though the terminal cannot be activated electrically nor even by the addition of α-latrotoxin. It might be that in the presence of diamide α-latrotoxin cannot interact with the terminal. However, we find that the toxin is still capable of resulting in a large decrease in the number of vesicles in the terminal as seen in EM.

2525 TRANSMITTER RELEASE STUDIES WITH DIAMIDE AT FROG NEUROMUSCULAR JUNCTION AND IN BRAIN SLICES. Patricia D. Wade*, Lawrence C. Fritz and E. Siegelvitz. Rockefeller University, New York, N. Y. 10021. Diamide, a sulfhydryl oxidizing agent, is known to dramatically increase the frequency of miniature endplate potentials at the frog neuromuscular junction in a Ca++-independent manner (Neuman, R. et al., Nature New Biology 231:120, 1971) similarly to a toxin from the black widow spider (Frontali, Werman, et al., Nature New Biology 233:120, 1971). Diamide also parallels α-latrotoxin in that following the waning of a maximal response further attempts to activate the terminal are unsuccessful. For example, upon electrical stimulation the evoked potential is blocked. Since toxin-treated terminals are empty of vesicles (Clark, A. et al., J. Cell Biol. 51, 1971), it is of interest to know how diamide-treated ones appear in the EM. Although not always entirely normal in appearance, diamide-treated terminals typically do contain vesicles, even though the terminal cannot be activated electrically nor even by the addition of α-latrotoxin. It might be that in the presence of diamide α-latrotoxin cannot interact with the terminal. However, we find that the toxin is still capable of resulting in a large decrease in the number of vesicles in the terminal as seen in EM.

2526 SPIKE AFTER HYPERPOLARIZATION AND TRANSMITTER RELEASE IN INTER-NEURON L10 OF APLYSIA. Rafiq Waziri. Dept. of Psychiatry, Univ. of Maryland School of Medicine, Baltimore, MD 21201. Interneuron L10, show that the hyperpolarizing phase of electrotonic potentials is diminished or abolished depending on the level of preganglionic stimulation (Christ & Nishi, J. Physiol. 213, 1971). We have tested the hypothesis that presynaptic α-receptors inhibit synaptic transmission at low frequencies that diazepam actually facilitates synaptic transmission. This effect is stereo-specific, 1-epinephrine (1-EPI) being more potent than α-epinephrine. The order of potency for the α-agonists is 1-NE, 1-epinephrine methyl tartrate, then α-agonists. We demonstrated that the α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture. Electrical field stimulation (3Hz, 20 volts, 5 msec. duration) was applied to and immediately following stimulation. 3H-NE and its deamination products 3H-DOPEG (100%) and 3H-DOMA (50%). Coapplication of tetraethyl amine blocks 3H-MOR uptake by 85% but does not affect spontaneous release of 3H-MOR. The alpha-adrenergic antagonists phenoxybenzamine, phentolamine and piperoxane all increase spontaneous release of 3H-MOR in a dose-dependent manner with the following order of potency: phenoxybenzamine > phentolamine > piperoxane. In conditioned preparations, phentolamine abolished stimulated release of 3H-MOR is inhibited by alpha-adrenergic agonists. This effect is stereospecific, 1-epinephrine (1-EPI) being more potent than α-epinephrine. The order of potency for the α-agonists is 1-NE, 1-epinephrine methyl tartrate, then α-agonists. We demonstrated that the α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture.

2527 ARE PRESYNAPTIC ALPHA-RECEPTORS INHIBITORY? S.D. Whitting*, D.H. McAffee, J.P. Hurn, and N. Wenger*. Div. of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010. Synaptic transmission is dependent upon Ca++ in most preparations. Recently, Hurn & McAffee (Science, in press) demonstrated that activation of postsynaptic α-receptors by a toxin from the black widow spider (α-latrotoxin) reduced synaptic transmission at low frequencies of preganglionic stimulation (Hurn & McAffee, J. Physiol. 213, 1971). We have tested the hypothesis that presynaptic α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture. The electrotonic potentials in L10, produced by action potentials of L10, show that the hyperpolarizing phase of electrotonic potentials is diminished or abolished depending on the level of induced hyperpolarization. Hyperpolarization of L10, which diminishes transmitter release, also diminish the spike after hyperpolarization in L10, and the hyperpolarizing phase of the electrotonic potential decreases slightly with the spike after hyperpolarization. Lowering of extracellular calcium concentration does not decrease the amplitude of the spike after hyperpolarization. These observations indicate that the α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture.

2528 ARE PRESYNAPTIC ALPHA-RECEPTORS INHIBITORY? S.D. Whitting*, D.H. McAffee, J.P. Hurn, and N. Wenger*. Div. of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010. Synaptic transmission is dependent upon Ca++ in most preparations. Recently, Hurn & McAffee (Science, in press) demonstrated that activation of postsynaptic α-receptors by a toxin from the black widow spider (α-latrotoxin) reduced synaptic transmission at low frequencies of preganglionic stimulation (Hurn & McAffee, J. Physiol. 213, 1971). We have tested the hypothesis that presynaptic α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture. The electrotonic potentials in L10, produced by action potentials of L10, show that the hyperpolarizing phase of electrotonic potentials is diminished or abolished depending on the level of induced hyperpolarization. Hyperpolarization of L10, which diminishes transmitter release, also diminish the spike after hyperpolarization in L10, and the hyperpolarizing phase of the electrotonic potential decreases slightly with the spike after hyperpolarization. Lowering of extracellular calcium concentration does not decrease the amplitude of the spike after hyperpolarization. These observations indicate that the α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture.

2529 ARE PRESYNAPTIC ALPHA-RECEPTORS INHIBITORY? S.D. Whitting*, D.H. McAffee, J.P. Hurn, and N. Wenger*. Div. of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010. Synaptic transmission is dependent upon Ca++ in most preparations. Recently, Hurn & McAffee (Science, in press) demonstrated that activation of postsynaptic α-receptors by a toxin from the black widow spider (α-latrotoxin) reduced synaptic transmission at low frequencies of preganglionic stimulation (Hurn & McAffee, J. Physiol. 213, 1971). We have tested the hypothesis that presynaptic α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture. The electrotonic potentials in L10, produced by action potentials of L10, show that the hyperpolarizing phase of electrotonic potentials is diminished or abolished depending on the level of induced hyperpolarization. Hyperpolarization of L10, which diminishes transmitter release, also diminish the spike after hyperpolarization in L10, and the hyperpolarizing phase of the electrotonic potential decreases slightly with the spike after hyperpolarization. Lowering of extracellular calcium concentration does not decrease the amplitude of the spike after hyperpolarization. These observations indicate that the α-receptors act to control presynaptic Ca++ influx by studying synaptic depression in the rat SCG when this organ is maintained in culture.
RELATIONSHIP OF HIGH ENERGY PHOSPHATES AND pH TO SYNAPTIC TRANSMISSION FAILURE DURING HYPOXIA IN THE IN VITRO HIPPOCAMPAL SLICE. Tim S. Whittingham and Peter Lipton. Dept. Physiology, Sch. Med., Univ. of Wisconsin, Madison, Wis. 53792.

In vivo brain function fails rapidly during hypoxia. Transverse slices (3mm) of guinea pig hippocampi are incubated in bicarbonate buffer maintained at a perfusing gas of O2:CO2 or O2:N2 to study hypoxic transmission failure in vitro. Field potentials are recorded from the dentate granule cell region following stimulation of the perforant path. If after changing from O2:CO2 to N2:CO2 there is approximately a 25% decrease in the evoked response, while complete transmission failure occurs in ~3%.

Increased extracellular potassium (K+e) and other depolarizing conditions all accelerate the rate of transmission failure during hypoxia, indicating that depolarization of neurons is leading to the hypoxic transmission failure. Two means by which this hypoxic depolarization could occur are: 1) Inhibition of the Na-K pump, and 2) Changes in cell membrane permeability. In either case it is important to establish whether decreased levels of ATP are responsible for the alteration in function.

Thus, experiments were done to determine if the cellular [ATP] falls rapidly enough during hypoxia to account for the decay in the evoked response. The ATP content of tissue slices analyzed for ATP and phosphocreatine (PCr) was predominantly composed of the dentate gyrus region in which transmission failure was monitored. In normal 4.4 mM K+ superfuse, the first noticeable decrease of the post-synaptic response occurred in ~ 215s. At this time [PCr] had fallen ~375 (from 2.55 to 0.8 mM) to O2:CO2 superfuse, the ATP had decreased ~15% (from 2151 to 1621umoles/mg protein.) When these same experiments are done at 3.4 mM K+, the first noticeable decrease occurs at ~30s, while at this time the decay of ATP is ~60% (from 460 to ~35 umoles/mg protein) and the [ATP] remains unchanged (~15.5 umoles/mg protein in control vs ~15 umoles/mg protein in control.) The fall in the second prior to the first decrease in the post-synaptic response is 3.4 mM K+ superfuse indicates that a decrease in [ATP] may well contribute to the decay in the evoked response during hypoxia.

Intracellular pH has been observed to decrease during hypoxia. This fall could result in Na pump inhibition or changes in the ionic atmosphere correlating pH with the evoked response during hypoxia. Experiments are currently being done and will be described.

Partially supported by NIH 1R01NS14175.01

SLOW SYNAPTIC MODULATION OF EXCITABILITY MEDIATED BY INACTIVATION OF CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN MYASTIC NEURONS OF GUINEA-PIG SMALL INTESTINE. J.D. Wood, P. Grafe* and C.J. Mayer*. Physiologisches Institut der Universität München, 8 Munich 2, GFR 8000.

We have previously reported the ability of lepinotarsin, a neurotoxin found in the hemolymph of Leptinotarsa haldemani (LPT-H), to mimic the effects of inhibiting electrotonic potentials (EPSPs) on the neuron. LPT-H is a type C neurotoxin which is heat labile and has a small effect on excitability in the absence of Ca++. In this study we have examined the ability of LPT-H (a 100 units/ml) to depolarize the neuron, to increase input resistance, repetitive spike discharge and results in prolonged hyperpolarizing after-potentials. We now report that the calcium antagonists Mg2+ and Mn2+ mimic all of the characteristics of the slow EPSP. When the neurons were superfused with solutions containing either 16 mM MgCl2 and 1 mM CaCl2, or 2.5 mM MnCl2, the input resistance increased, the membrane depolarized, hyperpolarizing after-potentials were abolished and repetitive spike discharge occurred. The organic calcium antagonists verapamil and D600 did not produce these effects. The effects of elevated Mg and Mn were reversibly followed by decrease in membrane excitability. We have previously studied the effects of elevated Mg and Mn on the relationship between external potassium concentration and the resting membrane potential in these cells. The results, when plotted on a logarithmic scale, showed a close fit to the Goldman equation and the slopes of the plots were significantly reduced when either 16 mM MgCl2 or 1 mM CaCl2 was added to the solutions. Other studies in our laboratory have shown that Mg2+ in other types of myenteric neurons and putative gial cells. We used intracellular current injection to control membrane potential, and the experiments were performed with solutions containing extracellular Ca++ or Mg++. The results indicate that the Ca++ found within the neuron may play a role in the excitability of these neurons. We found that the calcium antagonists Mg++ and Mn++ increased the input resistance, and low excitability. The calcium antagonists and 5-HT appeared to produce similar excitable membrane effects with the exception that Mg++ has a larger effect on the slow calcium-dependent gk systems. Therefore, our results suggest that the slow synaptic modulation of excitability in the tonic-type myenteric neurons involves a mechanism in which the primary activation of the neurotransmitter to calcium is involved with the activities of calcium for the calcium-dependent gk systems.

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TISSUE CULTURE
NEURITE PROMOTION IN CILIARY GANGLIONIC CULTURES. Ruben Adler and Silvio Varon. Dept. Biol., Sch. Med., UCSD, La Jolla, CA 92037.

We have previously reported that chick embryo ciliary ganglionic (CG) neurons will survive in monolayer cultures if supplied with either chick embryo extract or medium previously conditioned over chick heart cell cultures to serum. The two supplements, however, differed in their neurite-promoting effects, as embryo extracts elicited neurite growth only on collagen and HCM did so only on glycosaminoglycans (SAM) substrata (Varon et al, Brain Res., 1979, in press). We report here that HCM contains two distinct and separable agents. One is a trophic agent (HCM-CNTF), which supports the survival of CG neurons on either substrate. This agent does not seem to adsorb to either substrate, and may act directly on the cells. Its relationship to the CNTF which we have obtained from retinocul target tissues (Adler et al, Science, 1979, in press; Manthorpe et al, Trans. Am. Soc. Neurochem. 10: 76, 1979) has not yet been investigated. The CG culture assay previously developed for the eye-CNTF indicates that our HCM contains 10-20 trophic units/ml.

The second agent (HCM-NPF), first described by Collins (Proc. Natl. Acad. Sci. USA 75: 5210-5213, 1978), is presumably an acidic material which adsors to PORN but not to collagen surfaces, and confers to the substratum a neurite-promoting activity for CG neurons (from either HCM or embryonic extracts). Pre-exposure of PORN-coated dishes to serially diluted HCM, followed by seeding of CG neurons in the presence of HCM, allows us to assign to the original HCM an NPF activity of 3-6 unit/ml.

Neurite-promoting activity can be imparted to a PORN surface also by other means. Ciliary ganglion explants placed on untreated PORN or on PORN precoated with HCM-NPF, display no neuritic outgrowth unless CNTF is also provided. On the pre­ted PORN, CNTF-supported neuritic growth is radially and unrestricted. On untreated PORN, CNTF-supported neuritic outgrowth also occurs, but it extends only within the visible confines of some substratum-attached material (SAM), which originates within the explant and coats the surrounding PORN in widening circles. Thus, it appears that both the tissue of origin (ganglionic) and antigenic (muscle) are capable of providing supportive terrains for the trophic-driven elongation of a neurite. (Supported by NINCD grant NS-07606).

HORMONAL REGULATION OF GLIAL RELEASED PROTEIN IN CELL CULTURE. Alaric T. Arenander* and Jean de Vellis. Department of Anatomy and the Laboratory of Neural Plasticity, UCLA, Los Angeles, CA 90024.

Glia cells synthesize and release a variety of macromolecular factors including glycosaminoglycans, proteins (e.g., Nerve Growth Factor), glycoproteins and polypeptides, some of which are capable of influencing the morphological and/or biochemical differentiation of neurons or synapses. There has been little analysis of the range of potential protein factors or of the control mechanisms which modulate their production and release.

We report here that monolayers of rat clonal and primary glial cells release into the culture medium a reproducible, broad spectrum of soluble macromolecules. Furthermore, the number of these glial-released proteins (GRPs) are shown to be regulated in a specific manner by a variety of hormonal agents. Conditioned medium was collected from CG glioma cell monolayers after being incubated for 24 hr in serum-free medium and labelled during the last 6 hr with either [3H] or [14C]glucose. SDS-polyacrylamide gel electrophoresis of soluble GRPs revealed 20 major peaks of labelled protein distributed over a molecular weight (MW) range of 10,000 to 300,000. Gel radio-labelled patterns were reproducible both within and between experiments based on visual examination of gels, 1/14C isotope ratios and label reversal. Exposure of the cells to hydrocortisone (HC, 1-2 µM) for 24 hr altered the pattern of GRP in a selective and specific manner. Six major GRP peaks were consistently observed to either increase or decrease based on plots of isotope ratios. The effect of HC was observed at 0.02 µg/ml (5.4 x 10^-8 M) but not at 0.002 µg/ml. Treatment of cells with 17-β-oestradiol (2 µM) gave no observable change in pattern of GRP. Dibutyryl cyclic AMP (1 mM) and isoproterenol (10 µM) were also capable of selectively influencing the GRP patterns but in a manner different from HC. A number of the GRP peaks are glycoproteins based on metabolic labelling with [14C]glucosamine. Primary cultures of astroglias and oligodendroglia obtained from newborn rats generated a spectrum of GRP closely resembling that of the adult. Exposure to the high hormone HC treatment also influenced the GRP pattern from mixed glial cultures. Thus, both clonal and primary glial cultures released a wide range of proteins (GRP) that were under specific hormonal control. The origin and possible role in neuronal-glial coupling of GRPs is being investigated. Supported by DOE and USPHS.

COLD-INSOLUBLE GLOBULIN ENHANCES NEURITE OUTGROWTH FROM CULTURED NEURAL RETINA AGGREGATES. Rebecca M. Akers, Deane F. Mosher* and Jack E. Lillie*. Deps. of Zoology and Medicine, University of Wisconsin, Madison, WI 53706.

Aggregates of embryonic chick neural retina extend few neurites on plastic tissue culture dishes. However, pre-incubation of dishes with human cold-insoluble globulin (CIG) dramatically increases both the number and length of outgrowing neurites. Within 24 hours of plating on CIG-treated dishes, retinal aggregates elaborate elaborate, sparsely-branched processes which extend radially over the culture surface for several hundred microns. Enhancement of neurite outgrowth is evident on culture dishes treated with as little as 2.5 µg CIG (in 1 ml of medium); maximal effects are observed after pre-incubation of dishes with 25 µg CIG. Treatment of dishes with equivalent amounts of other serum proteins (BSA or fetuin) has no apparent effect on neurite outgrowth. CIG-treated dishes incubated with affinity-purified anti-CIG antibodies no longer support neurite outgrowth; aggregates plated on such dishes resemble those cultured on untreated plastic surfaces.

Neurites growing on CIG-treated substrata have a different morphology than neurites grown on polylysine (PLYS)-treated dishes. Like CIG, PLYS enhances neurite outgrowth from retinal aggregates. W hile the mean neurite outgrowth from CIG-grown aggregates is 3.7 times that from PLYS-grown dishes, neurites growing on PLYS adhere to the substratum along their entire length, whereas on CIG the neurites occur only at the growth cone. Although CIG and PLYS both provide favorable substrata for retinal neurite outgrowth, the interactions of neurites with these two substrata appear to be different.

Supported by grants from NSF (PCM 76-16878 A01) and ACS (BCB 255) to J.E.L. and from NIMH (HL 21604) to D.F.M.


Acetylcholine receptor (ACHR) and myosin are reciprocally regulated under a number of conditions. Generation increases ACHR and decreases myosin. In primary cultured muscle cells tetrodotoxin also increases ACHR and decreases myosin while electrical stimulation decreases ACHR and increases myosin. There has been little analysis of the range of potential protein factors or of the control mechanisms which modulate their production and release.

We observed a similar effect with a phosphodiesterase inhibitor. A number of phosphodiesterase inhibitors were found to elevate or decrease myosin content in primary cultures of chicken 1-day embryonic muscle. Using 125I-α-bungarotoxin binding to surface receptors as an index of ACHR content, caffeine and theophylline (10-7 M) were found to cause moderate increases in ACHR (25-35%). Papaverine (3 x 10^-7 M) increased levels by 50% while Ro20-1724 (3 X 10^-7 M) elevated receptor by over two fold. The effect of Ro20-1724 appeared to be largely due to an increased rate of receptor accumulation. The apparent rate constant of accumulation (kacc) increased from 32 cpm/h (125I-toxin binding sites) to 55 cpm/h while half life, as measured by loss of 125I-α-bungarotoxin from previously labeled cells, was unchanged. Under these conditions myosin heavy chains were extracted and quantitated by densitometry on acrylamide gels. After 48 hours of treatment myosin content was 11.2 ± 0.9 µg/plate in control and 5.6 ± 0.9 µg/plate in the Ro20-1724 treated cells. Pulse chase experiments with 125I-methionine indicated that increased degradation is primarily responsible for the decreased myosin content (control t1/2 = 72 h, Ro20-1724 t1/2 = 22 h). No measurable differences in DNA content were observed. To gain a better understanding of the mechanism of action of Ro20-1724, several cyclic nucleotides were tested. Dibutyryl cAMP (10^-5 M) was found to increase ACHR levels by 80% while dbcGMP (10^-3M) had little effect. Both dibAMP and 8BrcAMP increased kacc by 50% with little if any effect on half life. In contrast dibGMP decreased heavy chain myosin levels by 40% after 48 h of treatment. Similar treatment with dibAMP had little effect on myosin content. These results suggest that cAMP and cGMP each play a role in the reciprocal regulation of ACHR and myosin by different mechanisms - cAMP affecting ACHR at a synthetic or insertion level and cGMP influencing myosin degradation.

The specific binding of [3H]quinuclidinyl benzilate ([3H]QNB) in surface cultures of dissociated murine spinal cord (SC) cultures was previously reported to increase with maturation of the cultures (Brookes, Burt and Goldberg, Neurosci. Abstr. 4:590, 1978). To establish that this muscarinic receptor binding is neuronal, we have treated SC cultures with selectively neurotoxic amino acids and have assessed for the disappearance of [3H]QNB binding. The cultures were prepared by mechanical dissociation of spinal cords (including dorsal root ganglia and meninges) from 13-day-old mouse embryos. Cell proliferation was inhibited by application of 5-fluoro-2'-deoxyuridine after about 5 days of incubation. SC cultures at 2-7 weeks of incubation were exposed to monosodium glutamate (10^-3 M) for 3 hr. The fraction of [3H]QNB binding present immediately after exposure was 0.88 + 0.12 (mean + SEM, N=3). The fractions remaining 1, 2 and 4 days after exposure were 0.87 + 0.05 (N=4), 0.80 + 0.05 (N=2) and 0.81 + 0.02 (N=4), respectively. The mean control values of [3H]QNB binding were 125-175 femto per mg protein. The non-collagen protein content (Wallace and Parriot, Analyt. Biochem. 87, 1978) of the exposed cultures was 0.86 + 0.10 (N=6) of the control value (189 mg protein per culture) 4 days after exposure. The loss of binding was not increased by prolonging the glutamate exposure to 24 hr. This was in contrast to the effect of glutamate with exposures as short as 10 min. When [3H]glutamate tracer was included with cold glutamate (10^-3 M) in the culture medium, sample counts did not change after 24 hr and 48 hr, with no radioactivity still left on non-collagenic chromatograms using two solvent systems. The concentration-effect relationship for the action of glutamate was very steep. The mean fractional reduction of [3H]QNB binding observed at 10^-3 M was 0.91 + 0.14 (N=5). However, the loss of binding observed at 10^-1 M was not increased by further raising the concentration of glutamate. The fraction remaining after 3 hr exposure to glutamate (10^-3 M) was dependent on the maturity of the cultures at the time of exposure. Cultures exposed at 8, 12 and 19 days of incubation yielded 59%, 29%, and 16% respectively, of control binding. Kainic acid was only marginally more potent than monosodium glutamate. When SC cultures at 2-7 weeks of incubation were assayed 2 days after a 3 hr exposure to kainic acid (10^-3 M) and the remaining fractions of control or [3H]QNB binding were 0.83 + 0.09 (N=3) and 0.85 + 0.11 (N=3), respectively. The actions of glutamic and kainic acids were not influenced by the presence of 10% heat-inactivated horse serum in the culture medium. In conclusion, at least 89% of muscarinic receptor binding in mature SC cultures is associated with a sub-population of cells, presumably neurons, which is destroyed by the neurotoxic amino acid. This time course of receptor binding is measured in days. (Supported, in part, by USPHS Grant MH 3901L)


Primary cell cultures are a great potential as models for the study, particularly by biochemical methods, of many aspects of neuronal development which are currently rendered inaccessible by the complexity and heterogeneity of the intact brain. However interpretation of biochemical results must rest on clear knowledge of cellular composition in cultures based on reliable methods of cell identification.

In order to define and quantify the composition of our monolayer cultures of rodent cerebellum, we have used three types of markers: (a) Autoradiography of 3H-GABA uptake using specific GABA analogues to distinguish glial uptake from uptake into putative GABAergic neurons; (b) Established immunological markers for neurons, astrocytes, oligodendrocytes, and fibroblasts. (c) New monoclonal antibodies to cell populations in culture.

Cerebellar cultures have been found to contain at 6 days in vitro (DIV): granule neurons(>5% of total cells), inhibitory GABA neurons(<1%, probably stellate and basket cells), astrocytes(<15%), oligodendrocytes(<0.5%), and fibroblasts(<0.5%). Cerebellar glial cultures have also been prepared from the same starting cell suspension using different media these contain at 6 DIV over 85% astrocytes and small proportions of the other cell types.

Once cell composition can be defined in this way, it is possible to adjust conditions to favour particular cell types, such as the inhibitory neurons. Cultures of greater than 95% granule neurons can now be obtained, being the purest cultures of a single neuronal type yet available.

The availability of a range of culture systems of differing and known composition provides a sound basis for biochemical studies of cellular development.


Primary cell cultures were performed on skin fibroblasts from 6 patients with Huntington's Disease (HD), 6 normal control subjects (NC) and 6 'at risk' subjects. For growth studies, cells were plated at 1x10^6 cells/60mm plate in Waymouth media containing 20% FCS. Fifty percent of the plates were counted each day for 5 days in 3 separate experiments. HD fibroblasts grew to a significantly higher maximal cell density than NC or at risk fibroblasts. The 5th day cell density for HD cultures was 4.76 x 10^6 at 10^4 M and for NC cultures was 3.55 x 10^6 at 10^4 M and for at risk cultures was 2.7 x 10^6 at 10^4 M on days 2-7. The effect appeared to be due to a specific growth factor for HD fibroblasts. The growth factor concentration was 10% heat-inactivated horse serum in the culture media.

Once cell composition can be defined in this way, it is possible to adjust conditions to favour particular cell types, such as the inhibitory neurons. Cultures of greater than 95% granule neurons can now be obtained, being the purest cultures of a single neuronal type yet available.

The availability of a range of culture systems of differing and known composition provides a sound basis for biochemical studies of cellular development.


Organotypic cultures of adult rat SCG explants have been maintained in chemically defined media for periods of at least two weeks. Deheadnated ganglia from rats weighing 125-150 g were cut transversely into 300 µm sections and placed in culture on either a collagen or Millipore filter. Alternatively, the explants could be cultured in a serum-free medium (Eagle's MEM, 10 mM glucose, 100 units/ml nerve growth factor and antibiotics) supplemented with 1% bovine serum albumin (BSA). Significant outgrowth occurred when the medium was further supplemented with at least one of the following: insulin (1 µg/ml), 1-thyroxine (4 µg/ml), and hydrocortisone (0.5 µg/ml). On the collagen surface growth observed in the presence of BSA and all three hormones was comparable to that observed with a 10% Fetal Calf Serum (FCS) supplement. Increasing the FCS concentration to 20% or adding all three hormones plus 10% FCS did not alter this.

The explant outgrowths are long neuronal-like processes containing catecholamines but no detectable dopamine. After freeze-drying the culture assembly the entire explant body can be removed from either the collagen or Millipore filter leaving the outgrowth attached to the surface.

Outgrowth may be quantitated by measuring NADPH-dependent dehydrogenases in the growing processes. Glucose-6-phosphate dehydrogenase (G6PDH) was the most active of the four major NADPH generating enzymes in SCG explants. Activities of G6PDH, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase (6PGDH), and malate dehydrogenase were 150 ± 10, 78 ± 6, 14.3 ± 2.7, and 8.6 ± 1.5 pmole NADPH/min, respectively in FCS media at 37°C. This pattern differs from that in situ where isocitrate dehydrogenase is the most active NADP-dependent enzyme. 6-phosphogluconate dehydrogenase, except F6PDH, were higher in the presence of FCS than in explants cultured with BSA and hormone supplemented media. Both G6PDH and 6PGDH showed a trend of decreasing activity in the serum-free media. Highest activity was noted in the presence of all three hormones and decreased progressively in media supplemented solely with insulin, thyroxine or hydrocortisone.

Organotypic SCG explants have great potential to be a useful system to quantitate growth and growth promoting substances in neural tissue. (Supported by USPHS Grant NS-14726.)
ANALYSIS OF THE MECHANISM OF NEURITE TRANSECTION IN CULTURE WITH J.K. Engelhardt, K. Ishikawa*, and D.K. Katase*. Dept. of Neurology, USC School of Medicine, Los Angeles, CA 90033.

The developmental increase in skeletal muscle resting potential has been a common observation in embryonic tissue (Boethius & Oppenheim, J. Physiol., 174: 281-294, 1967) and in primary cultures (Fischbach et al., Brain Research, 110: 170, 1975). Our 2.7 mM K+ solution had the following composition in mM: NaCl 140, KCl 2.7, CaCl2 1.8, Glucose 11.7, HEPES 16.7, pH adjusted to 7.4 with NaOH. 5.4 and 10.8 mM K+ solutions were prepared by substituting NaCl with KCl. The experimental protocol consisted of removing a culture dish from the incubator and discarding the media. The dish was rinsed with the appropriate experimental solution, refilled and allowed to equilibrate for 10 minutes before resting potential measurements were attempted. Experiments were conducted at room temperature (20-24°C).

Resting potentials measured in 2.7 mM K+ increased from 2.7 ± 0.6 mV (mean ± SD, n = 10) on day 4 to 86.1 ± 4.2 mV on day 8, while resting potentials measured in 5.4 mM K+ increased from 51.7 ± 2.4 mV on day 4 to 91.5 ± 4.0 mV on day 8. The change in average resting potential measured in 10.8 mM K+ was statistically significant (51.7 ± 2.4 vs 71.5 ± 4.0). The change in resting potential on other ions in the system, ions that may be unique to these myelin-forming cells. This behavior is consistent with the well-described changes in acetylcholine receptors (AChR) redistribution which occurs during in-situ synaptogenesis (see below). The ACh sensitivity measured in the cell body or a series of low amplitude contractions or sidebands of mepp amplitudes. The ACh sensitivity outside the contact areas, which are usually not direct but result from shock waves and associated physical phenomena due to vaporizing substrate. These transections are not as accurate as previously believed and often show neurite separation at sites 3 to 5 µm distal or proximal from the laser impact area during the process. Scanning electron microscopy also reveals occasional residual processes below the resolution of LM at laser impact sites. Although it forms a mechanism for the peristaltic pumping of material through the long, attenuated cytoplasmic processes in myelin.

References:

Supported by grants from the NIH and the MDA.

PULSATILE MOVEMENTS OF SCHWANN CELLS IN VITRO STUDIED BY TIME-LAPSE CINEMICROGRAPHY. David S. Forman, William C. Shap, Jr., David A. Puche and Clarence D. Braddock*. Naval Medical Research Institute and Armed Forces Radiobiology Research Institute, BNA, Bethesda, Maryland 20014.

The development of pulsed laser microbeam transection techniques (Engelhardt, J. Cell. Physiol., 86: 505, 1975) and oligodendroglia in tissue culture display slow pulsatile movements which may be used to separate nerve fibers from non-nervous tissue. This technique for in vitro nerve transection has been reported here in combination with a laser-based microbeam specifically designed to test the effect of low [K+] in the bathing solution on the ability to observe a developmental increase in resting potential. Our tissue culture and electrophysiological methods have been described elsewhere (Engelhardt et al., Brain Research, 110: 170, 1975). Pulsatile transections were measured using conventional glass micropipette electrodes filled with 3 M KCl. Our 2.7 mM [K+] solution was observed with phase microscopy in a continuously perfused Dvorak-Stottler chamber at 36°C and photographed at 6 frames/minute. Most of the Schwann cells were elongated and bipolar with small, high refractile cell bodies. However, they often extend more than three processes, and can flatten against the substrate. The cultures were usually filmed for an hour, during which an average of 36% of the Schwann cells displayed intracellular pulsatile activity. The duration of these active periods varied from 1 to 7 minutes, with an average of 2.7 minutes. The quiescent interval between pulsatile periods also varied significantly (average ± SD, n = 10) from 1 to 17 minutes were measured between pulsations (average ± 2.4 minutes), but there were many longer pauses which extended beyond the ends of the filming sessions. The average cell had 4.0 periods of pulsatile activity per hour. Two types of pulsatile movements are classifiable: (a) simple (mepp) amplitude decreases in the cell body or a series of low amplitude contractions or sidebands of mepp amplitudes. The ACh sensitivity outside the contact areas, which are usually not direct but result from shock waves and associated physical phenomena due to vaporizing substrate. These transections are not as accurate as previously believed and often show neurite separation at sites 3 to 5 µm distal or proximal from the laser impact area during the process. Scanning electron microscopy also reveals occasional residual processes below the resolution of LM at laser impact sites. Although it forms a mechanism for the peristaltic pumping of material through the long, attenuated cytoplasmic processes in myelin.

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BIOSYNTHETIC REQUIREMENTS FOR NEURITE OUTGROWTH IN TISSUE CULTURE

Laboratory, University of Michigan, Ann Arbor, MI 48109

we examined the biosynthetic requirements for neurite outgrowth, and of glycoprotein biosynthesis (tunicamycin). Neurite outgrowth was quantitated using a morphological nerve growth index.

normal for several days following inhibition of protein synthesis, then times, completely inhibits neurite outgrowth. However, maintenance of CXM (100µg/ml), either at the time of explantation or at subsequent times, inhibits neurite outgrowth. This inhibition was not overcome either by the presence in the culture medium (at 5 µg/ml), resulted in a 70% inhibition of outgrowth after 5 days in vitro and greater than 90% inhibition at 12 days. These results indicate that the functioning of the cholera toxin pathway is necessary for neurite outgrowth.

Another treatment which was found to cause intact neurites to degenerate was continuous in the culture medium (at 5 µg/ml), resulted in a 70% inhibition of outgrowth after 5 days in vitro and greater than 90% inhibition at 12 days. These results indicate that the functioning of the cholera toxin pathway is necessary for neurite outgrowth.

The knowledge of ultrastructural and cytochemical characterizations of Schwann cells cultured from rat peripheral nerve: Characterization by electron microscopy and cytochemistry. Ward F. Odenwald*, Valerie Askanas, W. King Engel, Linda S. Carter*, Jane V. Lawrence*, NIM, Bethesda, MD 20025

Schwann cells were cultured from 3 day old rat sciatic nerves with techniques established in this laboratory for human nerve cultures. Six 1 mm nerve explants were placed on collagen-coated 35 mm petri dishes, and subsequent reexplantations were performed until the formation of a dense monolayer of cells was obtained. Cultures were fed with media as described (Neurology 25:58-67, 1975). For satisfactory growth, the cultures were processed for cytochemical and electron-microscopic (EM) EM studies were performed on areas of cultured schwann cells (CSC) preselected by light microscopy as described (Stain Technology 52: 249-254, 1977). The majority of CSC had characteristic membrane surface invaginations, ~25nm deep. Major differences between CSC and ASC were lack of a basement membrane and the presence of polarly oriented bundles of microfilaments with helical periodicity (~56nm) in the cytoplasma of the CSC.

Positive peroxidase-post-coupled DAB staining of concanavalin A (100 µg/ml) binding revealed the presence of α-D-glucoside and/or α-D-mannoside groups on the PM of CSC. Staining with a low concentration of DAB (0.1 µg/ml) of ConA had less affinity for the invaginations toward ConA, suggesting an increased density of receptors there. Omitting the ConA from the incubating medium prior to staining did not abolish staining. A-mercaptoethanol resulted in lack of characteristic staining. Horseradish peroxidase (HRP), employed as a cytochemical tracer, was added to the incubating medium (0.1 µg/ml) of ConA and showed a marked affinity for the invaginations toward ConA, suggesting an increased density of receptors there. Omitting the ConA from the incubating medium prior to staining did not abolish staining. A-mercaptoethanol resulted in lack of characteristic staining. 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AN α-NEROOTOXIN THAT INDUCES THE RAPID INTERNALIZATION OF FLUORESCENT α-BUNGAROTOXIN BOUND TO AUTONOMIC NEURONS. Peter Ravdin*, H. Neumark*, and T. Nisimura. Dept. of Biology, UCSB, La Jolla, CA 92037.

Chick ciliary ganglion neurons and sympathetic neurons both have a high affinity binding site for α-bungarotoxin (Bgt 3.2), but the toxin does not block acetylcholine (ACh) sensitivity on the neurons. We have previously shown that another α-toxin (Bgt 3.1) from the same snake venom does block ACh sensitivity on the neurons. We now report that Bgt 3.1 binds to dissociated embryonic muscle cultures. At present the relation­ship between the factor and the disease remains unclear since the toxin does not block ACh sensitivity on the neurons.

Dissociated neurons prepared from 8-12 day embryonic chick ciliary and sympathetic ganglia were grown in cell culture for 1-3 weeks with skeletal myotubes. High affinity Bgt 3.2 binding sites on the neurons were fluorescently labeled by incubating the cultures in 4 × 10⁻⁶ M tetramethyl rhodamine-conjugated Bgt 2.2 (R-2.2) for 1 hr at 37°C. Subsequent incubation with 10⁻⁷ M Bgt 3.1 at 37°C led to nearly complete loss of surface fluorescence within 0.5 hr with the concomitant appearance of numerous small foci of intense fluorescence throughout the interior of the neurons. This apparent internalization of bound R-2.2 was blocked by low temperature (4°C) or by preincubation of the developing neonatal anterior horn segments. The criteria for neuronal shape, and eventually the extrusion of the nuclei.

We have previously shown that another α-toxin (Bgt 3.1) from the same snake venom does block ACh sensitivity on the neurons. We now report that Bgt 3.1 binds to dissociated embryonic muscle cultures. At present the relation­ship between the factor and the disease remains unclear since the toxin does not block ACh sensitivity on the neurons.

2550

ANTINEURONAL IN VITRO ACTIVITY OF SERA FROM PATIENTS WITH AMYOTROPIC LATERAL SCLEROSIS. P. J. Ennis*, H. Bartfeld*, and H. Neumark*, (Spon: A. Hess), Dept. of Anatomy, CAMBRIDGE-Yale University Medical School, Piscataway, NJ 08854, and the Amyotrophic Lateral Sclerosis Research Center of St. Vincent's Hospital and Medical Center, NY, NY 10011.

Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset, neurological disorder of unknown etiology which is characterized pathologically by the degeneration of upper and lower motor neurons as well as neurons in the motor nuclei of the lower cranial nerves. Wolfgan and Myers reported a high proportion of sera from patients with this disease that was toxic to cultured anterior horn segments. The criteria for neuronal shape, and eventually the extrusion of the nuclei.

These data indicate that Bgt 3.1 causes two separate events: inhibition of neuronal ACh receptors and internalization of bound R-2.2. When R-2.2 labeled neurons were incubated with 10⁻⁷ M Bgt 2.2 or B. viridis 4.7.3. toxin before and during challenge with Bgt 3.1, internalization of the bound R-2.2 was blocked. Under these conditions Bgt 3.1 still produced complete inhibition of hagmophorically measured ACh sensitivity.

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α-Choline can be transported across cell membranes by high-affinity (κ <4 μM) and low-affinity (κ >4 μM) systems. High-affinity transport (HACA) is a sodium independent transport that is found in sympathetic neurons, ganglia, and glial cells. Recently, Barsig & Berg (Rev. Biol. 65:90, 1978) reported HACA in fibroblasts cultured from human skin. HACA in these fibroblasts had a K for γA of 1 μM similar to that observed in dissociated spinal cord cells in culture.

We examined HACA accumulation in normal human fibroblasts cultured from skin biopsy. 12-hours HACA accumulation was temperature dependent and linear with incubation time up to 6 min at 0.125 μl choline. Isosmotic replacement of Na with Li (154M) or sucrose (274M) severely reduced 12-hour HACA accumulation (by 70-90%). Preincubation with ouabain (10⁻⁶M) or dinitrophenol (10⁻⁴M), or replacement of Na with Li had little or no effect on subsequent 12-hour HACA accumulation. 12-hour HACA accumulation was hemicholinium-3 (HC-3) sensitive with pre-incubation in HC-3 at 37°C for 10 min. HC-3 at 10⁻⁶M was toxic. The IC₅₀ for HACA was about 4 μM, which compares favorably to that observed in brain synapses and avian fibroblasts.

These data indicate that HACA is a sodium independent transport for choline. Presently, we are investigating HACA in fibroblasts of patients with inherited disorders associated with a defect in a cholinergic etiology. (Supported by grants to D.K.R., R.H.R. & X.O.B. from the Dystonia Medical Research Foundation).


Chick embryo muscle cells grown in tissue culture contain two major molecular forms of acetylcholinesterase (AChE) having sedimentation coefficients of 11.5 and 7.1S. In order to study the cellular distribution of AChE forms in culture we have modified the complement and determined the total cellular content of AChE molecules located on the surface membrane of this type of cells. AChE is not removed by treatment with trypsin and can be extracted from the cell surface with neutral detergents. AChE is not removed by treatment with trypsin and can be extracted from the cell surface with neutral detergents.

To determine which molecular forms of AChE are located on the cell surface, cultures were pre-treated with BW284c51, a reversible water soluble inhibitor which does penetrate the cells to inactivate the intracellular enzyme. Analysis by velocity sedimentation of the forms protected from DFP by BW284c51 indicated that both the 11.5S and 7.1S forms of AChE are located on the muscle cell surface. Using DFP to irreversibly inhibit all AChE at different stages of muscle cell differentiation in culture we can demonstrate that: 1) the cells synthesize the surface AChE forms in vitro, 2) the rate of surface AChE deposition increases during the time of cell differentiation, 3) following treatment with DFP there is a partial recovery of the cell surface AChE in mature cultures, and 4) the surface AChE molecules have a half-life which is several times longer than that of another well-defined muscle cell surface protein, the acetylcholine receptor. During recovery from DFP treatment muscle cells in culture synthesize both the 11.5S and 7.1S AChE forms which are initially located inside the cells, and later give rise to the surface membrane fraction as well as to the secreted forms. In these studies we have not observed species differences between the 7.1S and 11.5S molecular forms. A model depicting the synthesis, transport, and turnover of these membrane bound AChE forms will be presented.

This research has been supported by the Muscular Dystrophy Association of America.
2552 DXEMETHASONE INDUCES BIOCHEMICAL DIFFERENTIATION IN CULTURED MURINE NEUROBLASTOMA. Dean Sandquist, Larry Williams*, Aga C. Blank*, Shail Sahai, and Terence H. Williams, Dept. of Anatomy, University of Iowa, Iowa City, Iowa 52242.

It has been observed previously that dexamethasone has a dose-dependent effect on morphological differentiation and growth inhibition in murine neuroblastoma (clone NBP) in vitro. We now report that dexamethasone also induces biochemical differentiation (as determined by increased dopamine content and tyrosine hydroxylase activity), and morphological differentiation (as determined by glyoxylic acid-induced histofluorescence microscopy). Dexamethasone content was determined by the method of Schmidt and Bhagavan (Brain Res., in press), while tyrosine hydroxylase was assayed according to Woytre et al. (Proc. Natl. Acad. Sci., 69:2241).

Cells treated for seven days with 25 μM dexamethasone in ethanol have more intense and uniform greenish-yellow catecholamine fluorescence than solvent-treated or untreated controls. Dopamine content increased from 3.7 ± 0.6 μg per mg protein (untreated controls) or 5.1 ± 0.5 (solvent-treated controls) to 11.2 ± 1.2 μg per mg protein after dexamethasone treatment. Tyrosine hydroxylase activity increased from 0.015 ± 0.003 nanomoles 14CO2 per hour per mg protein (untreated controls) or 0.10 ± 0.02 (solvent-treated controls) to 0.35 ± 0.04 nanomoles 14CO2 per hour per mg protein after five days of treatment with 125 μM dexamethasone. 200 μg/ml Ro 20-1724 (an inhibitor of cyclic AMP phosphodiesterase) increased tyrosine hydroxylase activity to 0.70 ± 0.04 for untreated cultures, and 2.9 ± 0.4 for cultures treated with 25 μM dexamethasone (a 25 μM dexamethasone in Tyrode solution). Thus, dexamethasone appears to induce both morphological and biochemical differentiation in cultured murine neuroblastoma. Since agents which increase cyclic AMP levels (such as Ro 20-1724) are known to inhibit dopamine synthesis and dopamine content and dopamine content were significantly different from both untreated and solvent-treated controls (P<0.001), dexamethasone augments dopamine synthesis.

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Cerebellar explants prepared from newborn mice were exposed to 10-110 μg/cc cytosine arabinoside (Ara-C), an inhibitor of DNA synthesis (Engelhardt et al., Brain Res. 126:172-175, 1977) and embryonic chick DBG in culture (Nicol, J. Biol. Chem. 261:12975-12977, 1977). Cultures were exposed to 1-10 μg/cc cytosine arabinoside (Ara-C), an inhibitor of DNA synthesis, for the first 5-9 days in vitro. In contrast to our previous results, dexamethasone appears to induce both morphological and biochemical differentiation in cultured murine neuroblastoma. Thus, dexamethasone augments dopamine synthesis and dopamine content did not differ in the two media. However, in the case of calf serum, the amine content did not differ in the two media.


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Explant cultures from fetal rat brain can be maintained for several weeks if exposed to a complete defined medium. Dexamethasone was added to the medium to enhance attachment of the explants to either collagen or poly-D-lysine. Attachment could also be enhanced by incubating in serum for the first 4-5 days in vitro and then switching to N1 medium. The outgrowth pattern and morphological appearance of cultures maintained in N1 medium for up to 4 weeks differed in several respects from identical brain regions grown in serum-supplemented medium. Regardless of the initial attachment conditions, cultures grown in N1 had fewer background cells and less flattening of the explant. The morphology of the neurons and their processes in phase optics appeared healthy, similar to neurons grown in serum. To distinguish different cell types, neurons were labeled with tetanus toxin and horseradish peroxidase. Octodendrocytes were labeled with rabbit anti-galactocerebroside, and astrocytes with rabbit anti-galactocerebroside. Neurons had action potentials either with a simple monophasic depolarization or with a complex polysomatic depolarization. The complex potentials were biphasic, as the amplitude of the depolarizing phase was similar to the amplitude of the repolarizing phase, but the latency of the depolarizing phase was longer than the latency of the repolarizing phase. The complex potentials were biphasic, as the amplitude of the depolarizing phase was similar to the amplitude of the repolarizing phase, but the latency of the depolarizing phase was longer than the latency of the repolarizing phase. Therefore, complex potentials were biphasic, as the amplitude of the depolarizing phase was similar to the amplitude of the repolarizing phase, but the latency of the depolarizing phase was longer than the latency of the repolarizing phase.
CHRONOTROPIC RESPONSES MEDIATED BY ALPHA- AND BETA-ADRENERGIC RECEPTORS ON CULTURED CARDIAC MUSCLE CELLS. Christopher Nelson Sinback, Jr. Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, MD, 20014.

Action potential rate, duration, and amplitude. Maximum diastolic potential, etc., were recorded with intracellular microelectrodes from embryonic hamster cardiac muscle cells in primary cell culture. Noradrenaline, isoproterenol, and acetylcholine were delivered by iontophoresis.

Noradrenaline, and not isoproterenol, elicited positive chronotropic responses (increased action potential rate and hence increased beat rate) by activation of alpha-adrenergic receptors, Alpha-adrenergic responses were due to a fast, latency less than 500 msec, brief, 5-10 sec, depolarization which was abolished when external sodium was replaced with choline. Alpha-adrenergic responses desensitized rapidly.

Isoproterenol, and not noradrenaline, elicited positive chronotropic responses by activation of beta-adrenergic receptors. Beta-adrenergic responses were characterized by a slow, latency longer than 3 sec, sustained, slow decrease in action potential frequency due to increased slope of the pacemaker potential. Beta-adrenergic responses did not desensitize Beta-adrenergic responses were abolished by propranolol. During beta-adrenergic responses maximum diastolic potential and action potential overshoot generally decreased, but in some cells maximum diastolic potential increased.

In contrast, we have shown that the rate of appearance of new acetylcholine receptors (AChR) at a bungarotoxin (b-ACh) accessible sites, the turnover of AChR, (total AChR accessible sites) and the size of the intracellular pool are nearly the same in both control and monovalent ionophore treated cells. Since the monovalent ionophores reportedly block the flow of membranes out of the golgi (Tarasoff, A. & Vassali, P. J. Cell Biol. 79:694 (1978)) our data suggests that there are at least two types of membrane vesicles or carriers derived from the golgi- one whose formation is inhibited by the monovalent ionophores(normally transporting secretory proteins such as AChE) and one whose formation is unaffected by the ionophores (normally transporting integral membrane proteins such as AChE).

Detailed histochemical and electron microscopic analysis of the AChE secretory process show that in control myotubes, AChE is focal and is present only in the Golgi region. After cycloheximide treatment (100 μg/ml for 2-3 hours) AChE reaction product can no longer be found in the golgi; after monovalent ionophore treatment, two classes of AChE containing membrane vesicles appear. One class is perinuclear and is not accessible to externally applied ferritin; one class is cytoplasmic and is accessible to externally applied ferritin.

Acetylcholine vesicles derive from the golgi within two hours after the addition of 4 x 10^{-5} M Migericin. The cytoplasmic vesicles appear to be amputated from the t-system and the golgi-derived vesicles break up into smaller vesicles which resemble the t-system in appearance. We propose that the AChE resides in the golgi and is then transported by vesicle to cytoplasmic channels or the t-system of muscle. Once in the t-system, the enzyme is in communication with the external bathing medium. Experiments are now in progress to visualize the route of AChE transport. (Supported by N.S. 13860)


Oligodendrocytes have been isolated from lamb brains employing the procedure developed in our laboratory (Schnaith, et al., Bio-phys. J., 21, 51, 1978). Immediately after isolation cells are suspended in Dulbecco's modified Eagle's medium supplemented with 10% horse serum, plated at a density of 2.5 x 10^6 cells/cm² and incubated in an incubator with 5% CO₂ and humidity at saturation. Freshly isolated cells exhibit surface staining, as viewed by indirect immunofluorescence, with anti-galactocerebroside (a generous gift of Dr. M. Rapport) and anti-sheep myelin basic protein antisera (a gift from Dr. A. Morohba). The pattern of staining with both antisera is patchy but the patches appear larger and more diffuse. Small, compact, branching cells, which are predominant, stain positively with anti-galactocerebroside but not with antimyelin basic protein. The positive staining for myelin basic protein is consistent with the notion that components of myelin remain attached to the cells during the process of isolation. Galactocerebroside, on the other hand, is a normal constituent of the oligodendrocytic membrane (Raff, et al., Nature, 274, 813, 1978). Cell kept in culture for 4 to 28 days. Cells of the first type, which are predominant, stain positively with antibody against GFAP and the size of the intracellular pool are nearly the same in both control and monovalent ionophore treated cells. Since the monovalent ionophores reportedly block the flow of membranes out of the golgi (Tarasoff, A. & Vassali, P. J. Cell Biol. 79:694 (1978)) our data suggests that there are at least two types of membrane vesicles or carriers derived from the golgi- one whose formation is inhibited by the monovalent ionophores(normally transporting secretory proteins such as AChE) and one whose formation is unaffected by the ionophores (normally transporting integral membrane proteins such as AChE).

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2560 ELECTRICAL PROPERTIES OF ANEURALLY CULTURED ADULT HUMAN MUSCLE

Albert J. Tahmoush, Gregory K. Bergay, Valeris Askenas, Phillip G. Nelson and W. King Engel. NIH, Bethesda, MD 20205

Histological, ultrastructural and biochemical studies of aneurally cultured diseased human muscle have proved useful in elucidating the pathogenesis of neuromuscular disorders. Electrophysiological studies of cultured diseased human muscle may also prove useful for investigating neuromuscular disorders characterized by electrical abnormalities. In order to define the electrical properties of the cultured adult muscle fibers and to compare their properties to normal innervated adult human muscle, six biopsies from five patients were cultured according to our established techniques (Neurol. 25:58-67, 1975). The individual cultures were examined daily by phase contrast microscopy. When the muscle fibers became well-differentiated and cross-striated, after 21-28 days of growth, they were selected for study.

Spontaneous, slow, tonic-like contractions were visible in only one culture.

For electrophysiological studies, the cultures were placed in a perspex chamber mounted on the stage of an inverted phase contrast microscope. They were maintained at 37°C in a water-saturated 95% air 5% CO2 atmosphere. Recording and stimulating electrodes were inserted intracellularly. The group values (Mean ± S.D) for 34 fibers from all 6 cultures were: a) Resting membrane potential (Vm) of 52.4 ± 6.6 mV. This was approximately 30 mV lower than the mean reported from several studies of adult innervated human skeletal muscle. b) Input resistance of 5.5 ± 3.4 MΩ. This value was much higher than those reported for adult muscle fibers. c) Only two of 34 fibers were electrically excitable at Vm. These fibers were present in the only culture in which spontaneous contractions were visible. d) When the fibers were hyperpolarized to 80 mV, an action potential could always be elicited. The threshold for excitation was 22.6 ± 8.7 mV and the action potential amplitude was 83.4 ± 24.6 mV.

These data indicate that aneurally cultured adult human muscle fibers are electrically excitable. The membrane characteristics are different from those of innervated adult human muscle in vivo. These studies demonstrate that the electrophysiological investigation of cultured adult human muscle may prove useful in analyzing the influence of neural factors on the electrical characteristics of normal muscle as well as the electrical characteristics of diseased muscle.

2561 EFFECT OF NERVE GROWTH FACTOR ON UPTAKE OF 3H-DOPAMINE AND SENSITIVITY TO 6-HYDROXYPENTYLAINE TOXICITY IN A HUMAN NEUROBLASTOMA CELL LINE. Evelyn Tiffany-Castiglioni and J. Regino Perez-Polo. Dept. Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

The dopamine-β-hydroxylase (DBH) cell line SH-SY5Y is selectively toxic to cells of sympathetic neuronal origin as well as to certain non-neuronal cell lines. Cytotoxicity has been linked to higher uptake of catecholamines by sensitive cells than by resistant cells. Ivermace growth factor (IGF) protects cells from 60% toxicity both in vivo and in vitro by an unknown mechanism. There are several possibilities which could contribute to IGF's protective effects: a) IGF decreases catecholamine synthesis, b) IGF decreases catecholamine binding, c) IGF decreases catecholamine uptake by the cell, or d) IGF stabilizes a cell's response to toxic levels of catecholamines.

We tested the hypothesis that NGF, which also protects the cells to a slight extent, acts in part by inhibiting the low capacity uptake system. To explore this possibility, cells were incubated in 25% NGF and 100 M IGF for 10 days. As expected, sensitivity to 6OHDA became less sensitive when treated with NGF. Uptake of 3H-dopamine (10-7 M) was significantly reduced in cultures treated with NGF in comparison to untreated cultures. These findings suggest that NGF decreases 3H-dopamine uptake and also change the cell's sensitivity to toxic levels of dopamine.
The addition of serum results in several problems, not the least of which is proliferation of nonneuronal cells. The eventual formation of a constant monolayer of nonneurons in the cultures limits their usefulness in neurobiological studies. Also, simplification of medium composition to recognizable essential elements would eliminate the complex and relatively undefined nature of serum. A mixture of defined ingredients (insulin, transferrin, progesterone, putrescine and selenium) has recently been formulated which supports proliferative growth of rat neuroblastoma cells in serum-free medium (J. E. Bottenstein and G. Sato, Proc. Natl. Acad. Sci. 76: 514, 1979).

In the present studies, dissociated embryonic chick dorsal root ganglionic cells were plated on collagen-coated culture dishes in medium containing 10% fetal calf serum. After allowing 48 hr for adequate cell adhesion, the cultures received fresh medium supplemented with 10% serum or serum-free defined medium (N2), which comprised insulin, transferrin, progesterone, putrescine and selenium. The N2 medium was also used to culture cells from a variety of chick embryo central nervous system tissues (optic lobe, neural retina, spinal cord, telencephalon), and fetal or neonatal rodent.

As with the chick sensory neurons, N2 supported the survival of fiber-bearing cells (features typical of cultured neurons), while proliferation and in large part survival of flat cells were always suppressed. This, then, may provide a general way to obtain purified subpopulations of process-bearing, neuronal-like cells. (Supported by NINDS grants NS-07606 and NS-12893.)

**Tissue Culture**

**2564**


Cultivation of neuronal cells in synthetic media routinely requires serum supplementation and, in several cases, trophic factors. The addition of serum results in several problems, not the least of which is proliferation of nonneuronal cells. The eventual formation of a constant monolayer of nonneurons in the cultures limits their usefulness in neurobiological studies. Also, simplification of medium composition to recognizable essential elements would eliminate the complex and relatively undefined nature of serum. A mixture of defined ingredients (insulin, transferrin, progesterone, putrescine and selenium) has recently been formulated which supports proliferative growth of rat neuroblastoma cells in serum-free medium (J. E. Bottenstein and G. Sato, Proc. Natl. Acad. Sci. 76: 514, 1979).

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**2565**


Embryonic sensory neurons from 8 to 10 day chick embryos have an absolute requirement for the nerve growth factor (NGF) to survive and grow fibers. We have shown that the inorganic cations magnesium and potassium are also necessary for the survival of these cells. On the other hand, calcium is not needed for the survival of these neurons, but is needed for these neurons to grow fibers. Our culture medium is simply Gey's balanced salts solution (GBS), (Amer. J. Cancer 27, 45-76, 1936) with various concentrations of NGF, 0.1 to 300 ng/ml, and a mixture of defined ingredients (insulin, transferrin, progesterone, putrescine and selenium). The N2 medium was also required in both media. The N2 medium selectively maintained the neurons and did not support proliferation or even survival of nearly all nonneuronal elements. In culture, the cells in N2 media was as good and eventually better than in serum-containing medium. After 6 days in N2 the cultures consisted almost entirely of neurons (>95%).

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Nerve growth factor (NGF) induced increased cell-to-plastic and cell-to-cell adhesiveness in F98 rat anaplastic glioma cells. Increased adhesiveness could be observed at NGF concentrations as low as 1 ng/ml. Since malignancy is often associated with a decrease in cellular adhesiveness, this could explain, at least in part, the decreased tumor growth rate observed in anaplastic glioma-bearing rats treated with NGF (S. Vinore and A. Koestner, J. Neuropath. Exp. Neurol. 37: 704, 1978; Fed. Proc. 30: 1269, 1979). Conditioned medium from the NGF-secreting tumor cells, C-6 rat glioma and S-180 mouse sarcoma, also increased the adhesiveness of these cells, but this response was unaffected by anti-NGF IgG. Conditioned medium also induced adhesiveness in PC12 rat pheochromocytoma cells, and this was reduced by anti-NGF IgG. Conditioned medium collected from C-6 cells that were pretreated with 17β-estradiol, which stimulates increased NGF secretion, induced the highest degree of adhesiveness observed in both F98 and PC12 cells and this was unaffected by anti-NGF IgG. Insulin, epidermal growth factor, anti-NGF IgG, cytochrome-C, bovine serum albumin, dibutyl-cAMP, and conditioned media from PC12 and IMR-32 human neuroblastoma cells did not increase adhesiveness. The factor inducing these effects is non-dialyzable, heat sensitive, and ammonium sulfate precipitable, and its secretion appears to be stimulated by 17β-estradiol. Supplementing conditioned medium with control medium produced an even greater increase in adhesiveness than did 100% conditioned medium, suggesting that factors depleted from control medium by growing cells also are necessary for maximum adhesiveness. As little as 15% conditioned medium reduced the NGF effect on adhesiveness.

The processes induced by NGF in PC12 cells in vitro were longer and more filamentous than those induced by conditioned media. PC12 cells in conditioned medium also appeared flatter than NGF-treated cells. In F98 cells, the processes induced by NGF take longer to appear than those induced by conditioned media. None of the in vitro morphological responses to conditioned media in PC12 or F98 cells were neutralized by anti-NGF IgG. The data indicate that both NGF and certain conditioned media increase adhesiveness and induce morphological differentiation in F98 anaplastic glioma and PC12 pheochromocytoma, and that factors in addition to and other than NGF are active in the conditioned media.

This work was supported by grant NS12325 and Biomedical Research Support Grant RR05402.
TROPHIC FUNCTION
ACCELERATED DEGRADATION OF JUNCTIONAL α-BUNGAROTOXIN-ACETYLCHOLINE LINE RECEPTOR COMPLEXES IN DENERVATED RAT DIAPHRAGM. R.G. Brett and S.G. Younkin (SPON: L.R. Younkin). Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

The AChR in innervated and 5-day denervated left hemidiaphragms were labelled in vivo by injecting 1.0 ng of 125I-αBT into the thoracic cavity of anaesthetized male Wistar rats and keeping them in a vertical position overnight. Virtually all animals survived this procedure which labelled about 35% of the AChR in the left hemidiaphragm with no observable histological differences between labelled and non-labelled hemidiaphragms (p < 0.0005 by Student's t test). The result of this experiment could be partially or completely due to a denervation-induced decrease in the rate of degradation of junctional AChR. We therefore measured toxin-receptor complexes in innervated and 5-day denervated left hemidiaphragms 1, 3, 5 and 8 days after the labelling procedure (i.e., 6, 8, 10 and 13 days after denervation). The loss of toxin from the endplate-containing area of innervated hemidiaphragms occurred with an apparent half-time of 11 days. The loss of toxin from the endplate-containing area of 5-day-denervated hemidiaphragms was much more rapid, occurring with an apparent half-time of 2 days (p < 0.005 by Student's t test). This result indicates that the normal metabolic stability of junctional toxin-receptor complexes is reduced during the period between 6 and 13 days after denervation. Because of the uncertainty inherent in receptor turnover in vivo, we cannot establish whether the increased rate of degradation accounts entirely for the decrease in toxin binding seen 5 days after nerve severance on the denervation-induced change in junctional toxin-receptor complexes was examined. The toxin-binding to areas of muscle near the point of nerve entry and areas far from the point of nerve entry (long stump) were compared in innervated and 2-, 5- and 8-day denervated hemidiaphragms. Animals were sacrificed 5 days after the labelling procedure (i.e., 7, 10 and 13 days after denervation) when virtually all of the extra-junctional toxin-receptor complexes had been degraded. The decrease in 8-125I-αBT bound to denervated hemidiaphragms occurred earlier in proximal than distal segments of the muscle.

Nerve denervation contributes with inactivity to the changes occurring in muscle after denervation. A. Congizzo and L. Lutzenberger*, Istituto di Fisiologia, Università di Firenze, Italy.

Overwhelming evidence indicates that factors different from nerve impulses are involved, together with inactivity, in the development of the muscle modifications occurring in muscle after denervation. One line of evidence is that partial denervation, which produces muscle inactivity less effective than denervation in producing spike resistance to tetrodotoxin and fibrillation or extrajunctional acetylcholine receptors. Lack of a hypothetical "neurotrophic" factor could be involved. Although the possible role of such a factor is represented by the release of products of nerve degeneration between the muscle fibres, we have now obtained data in support of the latter interpretation with experiments of partial denervation of rat EOL and soleus muscles whose nerves were in addition subjected to impulse conduction block with tetrodotoxin containing cuffs for 2-3 days. We found that junctional and extra-junctional spike resistance to tetrodotoxin (TTX) as well as an extra-junctional sensitivity to acetylcholine (ACh) developed virtually to the same extent in the denervated and in the adjacent innervated, but impulse-blocked fibres. This was in striking contrast with the behaviour of impulse-blocked fibres of control muscles not containing any nerve fibres which, at this early time, showed little or no membrane changes. These results appear difficult to reconcile with the hypothesis of "neurotrophic" factors contributing with activity to the control of muscle membrane properties since such factors should not be missing from the impulse-blocked but innervated fibres of the partially denervated muscles. The results are on the other hand consistent with the interpretation that nerve degeneration consequent to denervation sets free the action of some factors that then produce the high values of ACh hypersensitivity and TTX resistance.
### 2571 MEMBRANE PROPERTIES OF THE SKELETAL MUSCLE AFTER BLOCKADE AND RECOVERY OF FAST AXONAL TRANSPORT


A single subperineural injection of betahexotoxin (BTX, 9.3 pmoles) into the peroneal nerve of rats causes a block of fast axonal transport and subsequent membrane depolarization of the surface fiber of the extensor digitorum longus (extensor) muscle. The onset of membrane depolarization is dependent on the site of BTX injection - the more distal the site, the longer the onset (Neurosci. Abstr. 4:294, 1978). BTX injection was made in the mid-thigh region, and 1-6 weeks following the surgery, the loss and recovery of function and the changes in activity of AChE and CAT in fast-twitch SOL (SOL) and slow-twitch soleus (EDL) were compared with those in sciatic nerve segments (1 cm long), one proximal (PM) and one distal (DM) adjacent to the crush site. Activity values were calculated as activity/muscle or unit length (cm) of nerve. Within 24 hours after nerve crush, neuromuscular transmission was blocked. Maximal loss of AChE and CAT activity was seen by the end of the second week. AChE activity in EDL, SOL and DM was reduced to 15-25% of control while the loss of AChE in PN was 50%. CAT activity was reduced to 40% in EDL, 20% in SOL, 25% in DM and 50% of control in PN. During the third week, functional reinnervation of individual muscle fibers was observed and full innervation was reestablished by week 8. During this period, AChE activity in SOL recovered rapidly, with maximal enzyme activity at 4 weeks after crush (200% of control) returning to control values by 8 weeks. Thereafter, CAT activity remained stable and did not change significantly from control levels. The observed difference may in part result from the difference in muscle fiber types in these two muscles. (Supported by NIH Grant #NS-12430 and grant from the Muscular Dystrophy Association of America, Inc.)


The changes induced by loss and reestablishment of functional nerve supply may offer a suitable model for studying the mechanisms that control nerve and muscle enzymes such as acetylcholinesterase (AChE) and choline transferase (CAT). A difference in the rate of loss and recovery of these enzymes may give us insight into the mechanisms by which these enzymes are controlled by nerve and muscle. Therefore, these enzymes were measured in the mid-thigh region, and 1-6 weeks following the surgery, the loss and recovery of function and the changes in activity of AChE and CAT in fast-twitch SOL and slow-twitch EDL were compared with those in sciatic nerve segments (1 cm long), one proximal (PM) and one distal (DM) adjacent to the crush site. Activity values were calculated as activity/muscle or unit length (cm) of nerve. Within 24 hours after nerve crush, neuromuscular transmission was blocked. Maximal loss of AChE and CAT activity was seen by the end of the second week. AChE activity in EDL, SOL and DM was reduced to 15-25% of control while the loss of AChE in PN was 50%. CAT activity was reduced to 40% in EDL, 20% in SOL, 25% in DM and 50% of control in PN. During the third week, functional reinnervation of individual muscle fibers was observed and full innervation was reestablished by week 8. During this period, AChE activity in SOL recovered rapidly, with maximal enzyme activity at 4 weeks after crush (200% of control) returning to control values by 8 weeks. Thereafter, CAT activity remained stable and did not change significantly from control levels. The observed difference may in part result from the difference in muscle fiber types in these two muscles. (Supported by NIH Grant #NS-12430 and grant from the Muscular Dystrophy Association of America, Inc.)

### 2573 THE PROPERTIES OF WHOLE FDL MUSCLE AND OF INDIVIDUAL FDL MUSCLE UNITS AFTER CROSS-REINNERVATION BY SOLLE MUSCULES IN CAT.

R. St. Dum, R. E. Boegman, and J. Topal Lab. of Neural Control. NICCS, NIH, Bethesda, MD, 20020.

The properties of the heterogeneous "fast twitch" flexor digitorum longus (FDL) muscle and its use in locomotion, the membrane properties of the extensor muscles (which are not reinnervated by control levels for 3 weeks after a subperineural injection of BTX). The delayed recovery in RMP and of the physiological properties of the neuromuscular synapses were most likely a consequence of degenerative processes such as proliferation of Schwann cell (which is known to occur following denervation) interposed between pre- and postjunctional membrane. Apparently in spite of the recovery of the fast axoplasmic transport, the pattern of muscle recovery is similar to that seen after transection of nerve (Supported by USPHS Grants NS-10263, and funds from Muscular Dystrophy Association and the Paralyzed Veterans of America Inc.)

### 2574 BENEFICIAL EFFECT OF WATER-DEPRIVATION ON MUSCULAR DYSTROPHY OF THE CHICKEN.


Drugs that beneficially affect hereditary muscular dystrophy of the chicken. Although water-deprivation was shown previously to decrease the ability. Further studies showed that water-deprivation also...

It has previously been shown (Sutter et al., Soc. Neurosci. Abstr. 3:461, no. 1475(1977); J. Biol. Chem., in press) that sensory ganglion neurons from chick embryos have two distinct high-affinity binding sites for nerve growth factor (NGF) on their cell surfaces. One class of receptors (site I) had a Kd of about 10^{-11} M; the other (site II) had a Kd of about 10^{-9} M. Both steady-state and kinetic analyses showed these two classes of receptors did not interact in a negatively co-operative manner. The site II receptors were already present on the surface of sensory cells at day 4 of incubation, whereas the site I receptors first appeared between days 5 and 6. Several lines of evidence indicated that the neurite outgrowth response to NGF was mediated by site I receptors.

These studies were extended to the cells of the chick embryo sympathetic ganglion. The NGF receptors were measured using the specific binding of 125I-NGF as an assay. Steady-state analysis of binding to dissociated cells at 30°C revealed two distinct sites with Kd's of about 10^{-11} and 10^{-9} M. The kinetics of association and dissociation of NGF and its receptor appear similar to those seen with the sensory neurons. Sites I and II were about equally sensitive to inactivation or degradation by trypsin. No evidence for negatively co-operative interactions between the two sites was found. The development of the receptors has been followed, and both sites I and II were found on sympathetic cells from day 6.5 to day 20 of incubation. There were about 10 clones on many site II as site I receptors per cell (as measured by steady-state analysis at 37°C); this ratio did not change significantly from day 9 to day 15 of incubation. In culture, non-neuronal cells from sympathetic ganglia exhibited high levels of site II receptors, but site I receptors have not yet been detected on non-neuronal cells. The NGF receptors on chick sympathetic ganglion cells seem, in most if not all respects, to be similar to those found on chick sensory ganglion cells.


This present work reports a fast chemotactic response of dorsal root axons to β-Nerve Growth Factor (NGF) and attempts to determine the physiological events contributing in this response. A local gradient of NGF was applied, via a micro-perfusion system, to single growth cones of cultured chick dorsal root axons (8-13 days approximate embryonic age). All axons tested turned and began to grow up the NGF gradient within 9-21 min, without any detectable change in growth rate. This directed growth was observed when the micropipette source contained 2-50 biological units (BU) NGF/ml compared to 53 particles /µm^3 in awake animals kept in ambient noise. This oriented growth is not due to effects on growth rate, survival, fluid movement, or general response to any protein, but rather appears to be a chemotactic response to NGF which may involve saturable NGF receptors on the growth cone.

Dorsal root axons also turned and grew toward 1 µM sources of mono- and dibutyl cyclic adenosine monophosphate (c-AMP), phosphodiesterase inhibitors (caffeine, theophylline, Roche 20-1724), 1 µM cyclic adenosine monophosphate (c-AMP) in the presence of 10^{-7} M Roche 20-1724, and 20 mM calcium in the presence of the calcium ionophore A23187 (10^{-7} M). These results suggest that the physiological mediators of the chemotactic response to NGF may be increases in the cellular levels of c-AMP and/or c-CMP, and free calcium. Supported by NIH grants NS 12207 and NS 07044.
**Trophic Function**

**2579**

**NEUROTROPIC SUBSTANCE AS MEDIATOR FOR AXON-SHEATH CELL INTERACTION.** H. Mei Liu*, SPON. Igor Klare*. Brown University, Providence, Rhode Island. 20956.

The present study aimed at defining the neurotropic mechanism between axon and sheath cell by in vitro and chemical methods. Cultures of sheath cells (Schwann cells) were established from sciatic nerves of chick embryos after 16 days of incubation and fed with serum-free medium for three days. The conditioned medium was then assayed to block neurotropic activity of chick embryo dorsal root ganglia (DRG) and found to have nerve growth promoting activity (NGF action). Gel filtration of the conditioned medium with Sephadex G-200 revealed three major components with the NGF action. Affinity chromatography using Con-A Sepharose abolished the NGF activity in the conditioned medium while purified *β* NGF was not affected by the same treatment. These findings indicated that *β* NGF molecules secreted by sheath cells form a complex with the Con-A binding material in the conditioned medium. Chick embryo DRG placed at a distance of 2-3 mm away from explants of sheath cells showed accelerated neurite growth oriented towards the explants. Adhesion between neurites and sheath cells was observed. The cell recognition and adhesion appeared to be mediated by a Con-A binding, PAS and colloidal iron positive material on the sheath cell and axon surfaces. The location of Con-A binding material corresponded to *β* NGF molecules on the cell membrane as revealed by indirect immunofluorescent technique (incubation with rabbit antisera to *β* NGF followed by fluorescein-conjugated rabbit IgG). The above findings suggest that *β* NGF molecules synthesized by the sheath cells exist as a complex with glycoproteins on the cell membrane. The macromolecule, tentatively called "neurotropic substance" (NTS), is constantly shed into the extracellular environment as a result of membrane turnover. This result is in vivo vivo, in a diffuse gradient of NTS surrounding the sheath cells may be envisioned. The biological activity of NTS consists of twofold: one is a local action and involves cell recognition and adhesion between axon and sheath cell that is mediated by the glycoprotein molecules and the other is a well known NGF action of following transport of *β* NGF molecules from NTS to the receptor on the axonal membrane. The neurotropic substance appears to be the guiding principle in the organization of the peripheral nerve during embryogenesis and regeneration.

Supported in part by grants from the NIMH, NSF and Paralyzed Veterans of America. GJM is a MDA Post-doctoral Fellow.

**2580**

**PURIFICATION OF A SCIATIC NERVE PROTEIN HAVING TROPHIC INFLUENCES ON SKELETAL MUSCLE IN CULTURE.** G. J. Markenfelt and T. H. Oh, Dept. of Anatomy, Unv. of Maryland Sch. of Med., Baltimore, Maryland 21201.

It has long been known that the spinal motor nerve exerts trophic influences on the morphologic, physiologic and metabolic properties of skeletal muscle. Evidence has been accumulated to indicate that such trophic influences are mediated in part by a neutrally-derived trophic substance(s). Citrate-soluble chicken sciatic nerve protein was fractionated biochemically and added to neurally embryonic skeletal muscle cultures in order to identify the component(s) with neurotrophic activity. Biological activity of fractions was assessed by the ability of the fraction to enhance morphological development of muscle cells and to stimulate the incorporation of [14C]-leucine into muscle protein. A protein fraction expressing trophic activity was obtained by ion-exchange chromatography on diethylaminoethyl-cellulose followed by gel filtration on Sephadex G-100 superfine. This protein fraction, when added to mature, muscle cultures at 3-day intervals, maintained muscle fibers in a well-differentiated state for at least 5 weeks, a time when control cultures had degenerated completely. Characterization of the active fraction by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a single major protein with a molecular weight (Mg) of 84,000. At low concentrations, the protein migrated as a "doublet" which is thought to correspond to glycoprotein dimers. Analytical isoelectric focusing revealed that the active protein was acidic, focusing as four species with pI values of 5.74, 5.77, 5.92 and 6.15. Maximal stimulation of muscle protein synthesis was elicited by 20µg of active protein per ml. These data suggest that the trophic influences on muscle has been identified and purified.

**2581**


The mechanism of action of androgens on striated muscle is not understood. One approach to this problem is to study the production of specific proteins in response to androgen administration. We studied glucose 6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the oxidative pentose phosphate pathway, as a biochemical marker, in the hormone-sensitive liver and muscle of immature male rats. Sixteen hours after one i.p. injection of testosterone propionate (2.5 mg/100 g. body weight in DMSO), G6PD specific activity increased by 58% (Table). However, other androgens, e.g., fluoxymesterone and 5α-dihydrotestosterone, were without effect. These latter substances have a common character, viz., they cannot be aromatized to estrogen. On the other hand, androstenedione, a metabolite of testosterone which is a biologically inactive isomer, estradiol-17α, produced no effect. These results demonstrate that the conversion of testosterone to estradiol mediates the increased G6PD following testosterone injection. This phenomenon represents a direct effect of estrogen on muscle and suggests that "androgen aromatase" is involved in the mechanism of the action of androgens.

**2582**

**HYPERSENSITIVITY TO ACh IN INNERVATED MUSCLE FIBERS.** E. J. Munoz-Martinez, Jesus Quere* and Pedro Joseph-Hathar*, Deptos. de Neurociencias and Ingenieria Clinica, CIEA del IPN, Mexico 14, D.F.

Ingestion of the fruit of Tulildora (Karwinskia humboldtiana) produces flaccid paralysis and segmental demyelination of peripheral nerves in cats and rats. Demyelination leads to conduction block of the nerve and, consequently, to functional denervation when motor axons are affected. However, it cannot be excluded that paralysis results in some cases from axonal degeneration. We investigated whether the soleus muscles of rats treated with extracts from Tulildora showed the alterations which are found after denervation; the extracts were administered through a gastric probe. After a latent period of 3-5 weeks, rats treated with a single dose (1.5 gr/Kg) showed paralysis. The soleus muscles were then removed under anesthesia and individual muscle fibers were tested in vitro for sensitivity to ACh (iontophoresis). The end plates showed the usual sensitivity but 90% of the explored fibers (215) showed hypersensitivity outside of the end plates as in the case of denervation. However, only 30% of these fibers were functionally denervated as judged by lack of response to nerve stimulation; in one fully innervated muscle, 8% of the fibers were also hypersensitive to ACh. In contrast, forelimb muscles from the same treated rats did not show hypersensitivity. The same type of experiments were repeated in rats treated with a purified polypeptide compound isolated from the fruit of Tulildora and the results were essentially the same as those described above.

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**Experiment**

<table>
<thead>
<tr>
<th></th>
<th>GSPD</th>
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<td>Estradiol-17β</td>
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<tr>
<td>5α-Dihydrotestosterone + 4-hydroxysteroids</td>
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</table>

* p < 0.05

These results demonstrate that the conversion of testosterone to estradiol mediates the increased G6PD following testosterone injection. This phenomenon represents a direct effect of estrogen which is probably distinct from the general myotrophic actions of androgens. (Supported by MDA, Inc., and by the National ALS Foundation, Inc.)

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**2583**

**INTERACTION.** H. Mei Liu*. (SPON. Igor Klatzo). Brown University, Providence, Rhode Island. 20956.

The present study aimed at defining the neurotropic mechanism between axon and sheath cell by in vitro and chemical methods. Cultures of sheath cells (Schwann cells) were established from sciatic nerves of chick embryos after 16 days of incubation and fed with serum-free medium for three days. The conditioned medium was then assayed to block neurotropic activity of chick embryo dorsal root ganglia (DRG) and found to have nerve growth promoting activity (NGF action). Gel filtration of the conditioned medium with Sephadex G-200 revealed three major components with the NGF action. Affinity chromatography using Con-A Sepharose abolished the NGF activity in the conditioned medium while purified *β* NGF was not affected by the same treatment. These findings indicated that *β* NGF molecules secreted by sheath cells form a complex with the Con-A binding material in the conditioned medium. Chick embryo DRG placed at a distance of 2-3 mm away from explants of sheath cells showed accelerated neurite growth oriented towards the explants. Adhesion between neurites and sheath cells was observed. The cell recognition and adhesion appeared to be mediated by a Con-A binding, PAS and colloidal iron positive material on the sheath cell and axon surfaces. The location of Con-A binding material corresponded to *β* NGF molecules on the cell membrane as revealed by indirect immunofluorescent technique (incubation with rabbit antisera to *β* NGF followed by fluorescein-conjugated rabbit IgG). The above findings suggest that *β* NGF molecules synthesized by the sheath cells exist as a complex with glycoproteins on the cell membrane. The macromolecule, tentatively called "neurotropic substance" (NTS), is constantly shed into the extracellular environment as a result of membrane turnover. This result is in vivo vivo, in a diffuse gradient of NTS surrounding the sheath cells may be envisioned. The biological activity of NTS consists of twofold: one is a local action and involves cell recognition and adhesion between axon and sheath cell that is mediated by the glycoprotein molecules and the other is a well known NGF action of following transport of *β* NGF molecules from NTS to the receptor on the axonal membrane. The neurotropic substance appears to be the guiding principle in the organization of the peripheral nerve during embryogenesis and regeneration.

Supported in part by grants from the NIMH, NSF and Paralyzed Veterans of America. GJM is a MDA Post-doctoral Fellow.
2583 CELL PROLIFERATION IN DENERVATED MOUSE MUSCLE. Marjorie A. Murray* and Norman Robbins (SPON: Vernon Rowland) Dept. Anat., Sch. Med., Case Western Reserve Univ., Cleveland, OH 44106

Nerves exert control over cell division in many target tissues. In adult skeletal muscle, cell proliferation increases soon after nerve section, but it is unclear which cell types proliferate or whether the response is spatially related to degeneration of nerve terminals. Indeed, some authors suggest that early nerve degeneration cannot be connected to a cellular reaction at the endplate region.

In the present study, cell division in the extensor digitorum longus muscles of young adult mice was studied by injecting [3H]-thymidine (3H-TDR) after denervation. Scintillation counting of muscle homogonates showed that 3H-TDR incorporation into DNA in the denervated muscle was higher than in the sham operated muscle for the first week after nerve section, with the peak response at 4 days. Autoradiography revealed that most of the label was in cells extrinsic to muscle fiber basal laminae, although a few satellite cells were labeled. Some of the extrinsic cells had characteristics of fibroblasts, while others appeared to be macrophages. Although several cell types incorporated label, they were not preferentially located near certain structures such as small venules or nerve terminals. The muscle was not edematous and no polymorphonuclear leukocytes were seen.

In a series of reinervation experiments, scintillation counting during recovery showed that 90-95% of the label injected at 3 days did not remain in the muscle 6 weeks later. Several reports indicate that the number of satellite cells increases after denervation. In this study approximately 1% of the labeled cells were satellite cells as confirmed by electron microscopy. This small number is not surprising since these cells comprise only 2-4% of the nuclei in the tissue. Satellite cell increases may result either from budding off of a nucleus rather than mitosis. Experiments to test this hypothesis are under way.

The stimulus for cell proliferation after denervation seems to be a generalized signal throughout the muscle. Since the signal is reaching cells that are not synaptically connected to the nerve, it may be a secondary signal resulting from an earlier change in muscle fibers. Alternatively, cutting the nerve may alter primary mitotic influences that are normally present.

Supported by grants from the Muscular Dystrophy Association and by NIH AG-00795.

2584 PERSISTENCE IN DEGENERATING SCIATIC NERVE OF SUBSTANCES HAVING TROPHIC FUNCTION ON CULTURED MUSCLE. T. B. Oh, G. J. Markel* (SP: Vernon Rowland), P. G. Hatcher and A. A. Zalewski. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, Md. 21201 and NIH, Bethesda, Md., 20014.

It is well known that cholinergic innervation exerts trophic influences on skeletal muscle and that such influences are mediated in part by trophic substances. Evidence for the trophic substances has been derived from studies showing that a nerve trunk can be disconnected from its muscle target and yet continue to maintain the muscle. When soluble protein obtained from normal or 21-d denervated chicken sciatic nerves was added to chick embryonic muscle cultures, the incorporation of precursor into muscle protein was significantly greater than in controls. Addition of these proteins to muscle cultures markedly enhanced the rate of morphological maturation of muscle cells. By 6 d, cross-striated myotubes were present in cultures treated with normal or degenerating nerve protein, whereas control cultures showed thin immature myotubes. Under our culture conditions, control cultures began to degenerate after 2 w. By contrast, addition of normal or degenerating nerve protein to mature muscle cultures maintained these cultures for at least 4 w. Recently, we have identified and purified a protein (MW 84,000) from normal chicken sciatic nerves which has trophic influences on cultured muscle. (Oh, T. B. et al. Proc. Nat. Acad. Sci., in press.) In order to determine whether this protein persisted in degenerated nerves, we separately solubilized proteins from normal, 3-d or 21-d denervated chicken sciatic nerves and examined them for the ability to maintain normal or degenerating muscle cultures. The resulting gels showed that an 84,000 dalton protein was present in the degenerating nerves. The present study shows that soluble protein from normal or degenerating chicken sciatic nerves is equally effective in promoting synthesis, maturation and maintenance of cultured muscle and that the electrophoretic pattern of normal and degeneration nerves is very similar. These results suggest that the trophic substance which migrated in a manner identical to the trophically active, 84,000 dalton protein. We conclude that the in vivo changes in muscle following denervation are not due to the depletion of trophic substances following nerve transection. Instead, these results indicate that a trophic substance is still associated with the degenerating, distal nerve segment and possibly with non-neuromuscular cells in the peripheral connective tissues.

(Hatched Chick. J. D. Peduzzi* and W. J. Crossland (SPON: J. A. Hafels). Department of Anatomy, Wayne State University, School of Medicine, Detroit, MI 48201.)

Previous investigations of unilateral eye removal in newly hatched chicks have shown that the ectomammillary (EMM) central region to the removed eye is reduced in volume up to 50% when compared with the EMM contralateral to the unoperated eye. The ventral lateral geniculate nucleus (GLv) is also reduced in volume by a smaller magnitude (30%). The above findings for the EMM and ventral lateral geniculate nucleus (GLv) are consistent with previous observations for the observed reduction in nuclear volume: 1) cell death, 2) reduction of neuron volume and/or 3) arrest of neuron growth. To determine these possibilities, chicks were unilateralized 3 days prior to hatching and compared at birth with the affected and unaffected sides of the brain. Comparisons were also made with unoperated animals. The brains were embedded in paraffin and 10 µm thick sections were examined. Neurons containing nuclear DNA were traced using a camera lucida to obtain neuron cross-sectional areas and to determine neuron number.

There was a significant cell loss in both the EMM (30%) and GLv (20%) by the end of the first post-operative week. The neuronal loss remained constant in both affected nuclei despite an additional 10 week survival time. In the GLv the cell loss was greater in the neuropil lamina (25%) than in the internal lamina (15%). Measurements of neuronal cross-sectional areas revealed a 20% reduction on the affected sides in both nuclei which was apparent by the end of the first week. Changes in cell size paralleled changes in cell number. Neuronal atrophy remained constant in the remaining neurons for at least 12 weeks after unilateral eye removal. In the GLv neuropil lamina were more severely reduced in area (30%) than neurons in the internal lamina (35%).

Although the correlation between the degree of nucleus of volume reduction following eye removal, the EMM and GLv neuropil lamina showed marked similarities. In both sites we have demonstrated that neuronal cell size is not necessarily correlated with cell number. The latter observation seems particularly interesting in view of the relatively few studies of anterograde transneuronal degeneration which have quantified cell loss.

(Supported by Grant EY-01796.)


The presence of a 2-3 cm. nerve stump retards changes in the number of acetylcholine receptors which occur after denervation (Uchitel and Robbins, Br. Res. 153: 539. 1978). In order to determine whether the nerve stump effect depends on systemic factors in the present.

Supported by grants from the Muscular Dystrophy Association and by NIH AG-00795.
TROPHIC EFFECTS OF MUSCLE ON NERVE II. ACETYLCHOLINE (ACh) SENSITIVITY AND RELEASE. Guillermo Pillar, Jeremy Tuttle*, and Ken Vacca*. Physiology Section, Biological Sciences Group, UConn, Storrs, CT 06268.

During normal in vivo development, ciliary neurons do not form infraganglionic, preganglionic synapses. However, embryonic ciliary ganglion cells form contacts upon themselves having typical synaptic ultrastructure during the initial two weeks in culture. These neuronal synapses are non-functional, due either to a lack of sensitivity to ACh or to the inability to release ACh at these synapses. These phenomena were further examined by 1) testing cultures of these neurons for the release of ACh; 2) examining other culture conditions which might lead to the retention or induction of membrane sensitivity to transmitter. 1) Ciliary ganglion neurons take up and convert into ACh (3H-Ch) from the medium and convert up to 50% of the transported 3H-Ch into 4-ACh. Also the 4-ACh synthesized is primarily derived from 3H-Ch taken up by a high-dependent high-affinity process. Cultures were then tested for 4-ACh release in response high [45], depolarization. In all cases, depolarization caused the release of a significant proportion of the neuronal 4-ACh into the media. However, the ACh released by neurons cultured alone was only 50-60% Ca++-dependent, with the remainder not requiring extracellular Ca++. Thus, the neurons cultured under conditions which seem to favor interneuronal synaptogenesis are capable of ACh release and the synapses formed are ineffective due to a lack of sensitivity to ACh. 2) Most ciliary ganglion neurons from ST 32 embryos lack sensitivity to iontophoretically applied ACh, and the synapses formed are ineffective due to a lack of sensitivity to ACh. 3) The presence of descending serotonergic fibers in the lumbar region of the frog cord suggested that the higher rate of 5HT uptake by the CM may be due to the presence of descending serotonergic nerve fibers ending in the CM. To test this, lesioning was performed by transversely sectioning the cord at the level of the last spinal root and a period of 10 days was allowed for degeneration of nerve fibers. At the end of this period a significant decrease in the rate of 3H-5HT uptake was observed in the CM. Intraventricular injection of 50µg of 5,6-dihydroxytryptamine, which results in selective destruction of serotonergic terminal, and allows the degeneration of 3H-5HT uptake to occur, also resulted in a decrease in 5HT uptake by the CM with little effect being seen in the more caudal portions of the FT. These observations would suggest that the presence of descending serotonergic fibers in the CM modulates glial transport of 5HT. The specificity of this modulation is under investigation.

Supported by PHS Grant NS11253, NHLBI Grant HL-58804, and NCI Grants CA18577 & CA17701 & PM08-096S, CONACYT, Mexico.
A trophic action of the sensory nerves of the cornea and their target tissue, the epithelium, was first suggested by Magendie in 1824. However, convincing evidence for this type of interaction has not been obtained. The present studies show that deafferentation produces functional as well as morphological alterations in both the corneal tissue and the epithelium.

Radiofrequency thermocoagulation, delivered by an electrode inserted through the soft palaee, was used to produce lesions in the medial portion of the trigeminal ganglion in anesthetized albino rabbits. Although there was no behavioral response to punctate mechanical stimulation of the cornea following the lesion, normal blinking (4 to 5/hour) was retained. Nerve degeneration was confirmed by electron microscopy. Slit-lamp observations revealed that the corneal surface was free from defects. Permeability in the denervated corneas was found to be significantly greater (p<.005, t=11) when determined by measurement of aqueous humor fluorescence 30 min after the instillation of 5% fluorescein.

Re-epithelialization of a 4 mm centrally placed ablation was examined in 13 animals. After 40 hr all control corneas were epithelialized, but the denervated corneas were only 72% covered. Complete counts of mitotic figures were made in 6 mm diam. central corneal buttons and stained by the orcein method. The denervated corneas contained significantly fewer mitoses (p<.001).

Scanning microscopy of the denervated epithelium has revealed alterations in structures responsible for the maintenance of cell shape and adhesion. Transmission microscopy in 6 rabbits showed that the cytoplasmic density of the epithelial cells decreased due to a loss of tonofilaments. Both hemidesmosomes and desmosomes stained less densely; in addition a decrease in number and size of the hemidesmosomes was noted. Scanning microscopy showed an abnormal desquamation of the surface cells many of which had lost microvilli and were smooth. In conclusion, it is shown here that the sensory innervation of the corneal epithelium is important to the maintenance of its tissue properties. (Supported by NIH Grant EY 02108 and Swiss Foundation Bio. and Med. Res.).

This "sodium response" (i.e., accumulation in the absence of NGF) can produce a substantial stimulation of neurite outgrowth in both sensory and sympathetic ganglia. Nicotine in concentration ranges of 35-500 µg/ml was tested on 8 day chick trigeminal, dorsal root and sympathetic ganglia with the explant method on collagen-coated Falcon culture dishes. They were cultured in Dulbecco HEN supplemented with 10% calf serum. Nicotine in concentration ranges of 80-250 µg/ml at a pH range of 6.8-7.2. Preliminary evidence suggests that sympathetic ganglia are more responsive to nicotine than either trigeminal or dorsal root ganglia. Studies are in progress to determine the mechanisms behind this effect.

Supported in part by the University of Kentucky Tobacco and Health Research Institute, Project Number KTHB 2411.

Miniature endplate potentials (MEPPs) were examined in posterior latissimus dorsi (PLD) muscles of normal (line 412) and dystrophic (line 413) chickens from 6 to 12 week ex ovo. At 23° C the MEPP amplitude in surface fibers of normal PLD muscle (0.37 ± 0.02 mV) was greater (P< 0.01) than dystrophic (0.26 ± 0.02 mV) whereas the frequency of MEPPs in normal fibers (0.23 ± 0.02 sec⁻¹) was similar to that seen in dystrophic fibers (0.24 ± 0.02 sec⁻¹). Increasing the temperature to 40° C produced a unimodal exponential increase in MEPP frequency in both normal and dystrophic fibers with a Q₁₀ between 2 and 3 and energy of activation between 30-40 kcal. The difference in MEPP amplitudes were sustained at 40° C (0.40 ± 0.03 mV in normal vs. 0.39 ± 0.03 mV in dystrophic; P< 0.05), while the MEPP frequencies remained indistinguishable (0.93 ± 0.20 sec⁻¹ in normal vs. 2.44 ± 1.04 in dystrophic). MEPPs from fibers of both lines had similar rise times at both 23° C (3.32 ± 0.16 msec in normal vs. 3.57 ± 0.30 msec in dystrophic) and at 40° C (0.42 ± 0.12 in normal vs. 0.45 ± 0.10 in dystrophic). Half-decay times of MEPPs in both lines were also similar at 23° C (6.81 ± 0.24 msec in normal vs. 7.79 ± 0.58 msec in dystrophic) and at 40° C (0.79 ± 0.06 msec in normal vs. 0.89 ± 0.13 msec in dystrophic). At 23° C input resistance at the endplate region in normal fibers (0.44 ± 0.03 MΩ) was not significantly different from dystrophic fibers (0.41 ± 0.06 MΩ). Addition of neostigmine (3 µM) to the bathing medium or treatment with diisopropylphosphorofluoridate (1 mM) at 23° C produced a similar degree of potentiation of MEPP amplitude in normal and dystrophic fibers. It appears unlikely that the observed differences in the amplitudes of MEPPs in normal and dystrophic fibers can be attributed to differences in input resistance or junctional acetylcholinesterase alone. It seems probable that the decrease in MEPP amplitude in dystrophic fibers is due to either some alteration in the transmitter release process as suggested previously (Exp. Neurol. 63:235, 1978) or to a reduced access of acetylcholine to the receptor site since it has been shown that junctional acetylcholine sensitivities of normal (line 200) and dystrophic (line 304) chickens are similar (Exp. Neurol. 42:21, 1974). In addition, at temperatures from 18 to 41° C the MEPP frequency in PLD muscle fibers of both normal and dystrophic chickens fails to show the bimodal response to temperature increases seen in some other vertebrate species (J. Physiol. 132:650, 1956). (Supported in part by USPHS Grant NS-12063 and a grant from the Muscular Dystrophy Association of America.)
VISION
**2596**

**SPATIAL AND TEMPORAL PROPERTIES OF RECEPTIVE FIELDS IN MONKEY AND CAT VISUAL CORTEX.** Dume G. Albrecht, Lisa G. Thorell* and Russell L. De Valois*. Department of Psychology, University of California, Berkeley CA 94720.

The spatial and temporal variations are the two most important aspects of a visual stimulus with respect to evoking a response in a visual cortical neuron in area 17. The structure of the receptive field demands a specific spatial and temporal distribution of light. To quantify these space-time contingencies, one would like to have a method of analysis which produces a concise, accurate, general description. Use of spatial-temporal sinusoidal grating patterns, within the context of linear systems analysis, can potentially provide such a description. In this study we used such a method of analysis to measure (a) the spatial tuning, (b) the temporal tuning, and (c) the interaction between two.

With respect to the spatial tuning, the results show that most of the cells have a band pass characteristic; each cell attenuates its response to both high and low spatial frequencies. The average width of the band at half the maximum response (averaged across all of the cells) is 1.2 octaves. There is, however, a great deal of variability in this regard from cell to cell; the narrowest bandwidth was 0.5 octaves and the broadest was greater than 2.5 octaves. There is also a great deal of variability in the respect to the locus of the peak of the spatial tuning function.

With respect to the temporal tuning function, while most of the cells (75%) did not respond to both low and high temporal frequencies, the tuning is extremely broad (in comparison to the spatial tuning). There is a great deal of variability from cell to cell with respect to the width of the tuning function and the locus of the peak. The mean bandwidth for the entire sample was 3 octaves and the range extended from 2 to 6 octaves. The mean peak for the entire sample was 3 Hertz and the range extended from 0.5 Hertz to 16 Hertz. Somewhat surprisingly, there were no obvious differences in the temporal properties of simple vs complex cells. Correlation (across cells) of the peak of the spatial tuning function with the peak of the temporal tuning function revealed a very low negative correlation (r = -0.2).

Human psychophysical experiments have shown a clear interaction between spatial and temporal variables of a visual stimulus. Parameter examinations of these two variables for area 17 cells showed that for the vast majority of cells no such interaction existed. That is, for most cells the spatial tuning remains invariant as a function of temporal frequency. A few cells, however, showed a systematic interaction between these two variables.

**2597**

**REGIONS "U" AND "V" OF CAT VISUAL CORTEX.** Duane G. Albrecht and D. Sanides*, Dept. Neurobiology, SDI Biochemistry, 3400 Geltingen, TX.

In normal adult cats three regions with callosal projections (callosal islands) have been identified at the lateral limit of area 10 by anterograde and retrograde axonal tracing techniques. Defective fields of single units and multunits have been recorded in the callosal islands and were found to reach an extremely large size. Their centers lay either on the vertical meridian or 10° to 20° in the contralateral hemifield. However similar as in other regions with callosal input the fields reached across the vertical meridian and often considerably into the ipsilateral hemifield. The callosal islands were wedged between acallosal parts of lateral area 10 which represent the periphery of the contralateral visual field (peripheral islands). Thalamic input to these different two parts of lateral area 10 has been identified by retrograde tracing with peroxidase. The periphery islands have been found to receive heavy input from the main part of the lateral geniculate nucleus (LC+) and light input from the medial interlaminar nucleus (LC-). In both nuclei it was restricted to portions with peripheral retinal input. The callosal islands have been found to receive a peripheral visual input from the LC+, and a rather heavy projection from portions of the LGN representing the contralateral retina. Prolongation of the LC- projection to the ipsilateral retina. It thus appears that the extremely large receptive fields in the callosal islands of lateral area 10 are produced by convergingafferent fibers with widespread retinal input.

In some animals large receptive fields covering nearly the entire visual field were found also at the representation of the vertical meridian in area 10. The pattern of thalamic connections of these medial parts of area 10 resembled that of the callosal islands in lateral area 10.

The callosal regions in area 10 fulfill the criteria on a selective basis that they are a representation of most of the visual field. Unlike the remainder of area 10 and other visual cortical areas the representation is not or only poorly retinotopically organized.

**2598**


We have used the steady state visual evoked potential (VEP) to study the changes in both low and high frequency bands following the very brief periods (e.g. 10 sec) in the dark. Under our recording conditions, the synchronous response to uniform field flicker and counterflicker following the steady state visual evoked potential (VEP) is 3 octaves and the range extended from 2 to 6 octaves. The mean peak for the entire sample was 3 Hertz and the range extended from 0.5 Hertz to 16 Hertz. Somewhat surprisingly, there were no obvious differences in the temporal properties of simple vs complex cells. Correlation (across cells) of the peak of the spatial tuning function with the peak of the temporal tuning function revealed a very low negative correlation (r = -0.2).

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**2599**

**SMOOTH PURSUIT EYE MOVEMENTS.** A. Terry Bahill and B. Todd Troost, Biomedical Engineering, Carnegie-Mellon University, Pittsburgh, PA 15213.

The easiest target to track with smooth pursuit eye movements is one that is moving sinusoidally. To track such a target human uses the saccadic system, the smooth pursuit system, and high level prediction processes. In order to limit our study and eliminate the prediction process we used unpredictable target motions. Gaussian white noise was too confusing and inconsistent; we used an extremely low frequency sine wave with a series of constant velocity ramps which each of which has an unpredictable velocity and duration. The bandwidth of the target was also important; a bandwidth of one hertz was optimal.

The recorded eye movements consisted of a combination of smooth pursuit and saccades--dual mode tracking. We modified this record by removing all of the saccades to yield a cumulative smooth pursuit eye movement record--single mode tracking. We visually inspected these time domain records, but did all of our data analysis in the frequency domain.

Fatigued subjects, and patients with progressive supranuclear palsy had large differences between the single mode and dual mode transfer functions, particularly around one hertz. Patients with unilateral posterior hemispheric disease had smooth pursuit movements in only one direction which caused a large low frequency gain for the single mode transfer function and a very low value for the coherence function at low frequencies.

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In a series of penetrations through foveal striate cortex in alert behaving macaque monkeys, we noticed that the first encountered striate cells typically had a preference for lines oriented at 45° to horizontal and vertical. This was a somewhat surprising result in view of Mansfield's (1974) earlier report of an anisotropy in alert and geniculocortical pathways in foveal striate cells. Review of our own data from marked penetrations in acute, anesthetized animals suggested that the horizontal/vertical anisotropy was in fact most prevalent in middle and lower layers of striate cortex.

In order to pursue this issue more systematically, we have been performing a series of penetrations aimed from the foveal surface to white matter in penetrations at various angles to the cortical layers. The angle of our electrode is determined by the geometry of the monkey's skull. Variations in recording angle within a given hemisphere are thus primarily dependent on the contours of the cortical surface. To avoid bias in our test procedure we routinely present a series of standard orientations to every cell and use computer plotted histograms as a measure of response magnitude.

In a typical penetration through foveal striate cortex, the first encountered cells have orientation preferences within about 15° of diagonal. Subsequent cells prefer orientations close to this, with slight progressions in either a clockwise or counterclockwise direction until the middle of layer 4, when orientation tuning becomes much broader or disappears completely. Toward the lower part of layer 4 the orientation suddenly jumps by 45° or more and then remains about the same until we enter white matter.

The most parsimonious interpretation of our data is that there are alternating horizontal and vertical orientation dominance columns in foveal striate cortex. Each column must necessarily consist of 2 subregions, one responsible for clockwise rotation, the other for counterclockwise rotation. Counterclockwise rotation from vertical to horizontal and vertical to horizontal rotation from horizontal would both lead to the same limit, namely 45°, suggesting that 2 such subregions should lie adjacent to one another. Similarly, horizontal to diagonal and diagonal to horizontal subregions should be also adjacent. The anisotropy for diagonal orientations in upper layer cells bears some resemblance to the binocularity of upper layer cells (Hubel and Wiesel, 1968), except that inhibitory mechanisms would appear to play a greater role in generating diagonal orientations, and two different diagonals must be generated instead of a single cyclopean eye.

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2604 EFFECTS OF INFERTEMPORAL COOLING ON PERFORMANCE OF A VISUAL RECOGNITION TASK. Richard H. Bauer, John P. Jervey* and Joaquin M. Fuster. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Ablation studies in the monkey have implicated the infero-temporal cortex (area TE) in learning and retention of visual discriminations. We therefore examined the effects of reversible lesions on the retention of visual stimuli. For this study the stimuli were circles of red and green light projected on a panel. A trial required the animal to perform two successive instrumental responses. The first occurred immediately upon presentation of one color, the sample, which initiated the trial. The second response occurred at the end of the delay, when the two colors appeared simultaneously and the animal had to select the one that matched the sample. Correct choices were rewarded. The sample color and the position of the matching stimulus were changed randomly between trials. Cryogenic brain probes were implanted bilaterally in three monkeys trained to perform the task. The probes conformed to the convexity of the temporal lobe and were epidermally covered, major portion—about 1 cm²—of TE cortex. Cortical temperature was controlled by implanted thermistors. Bilateral inferotemporal cooling to 20°C produced the following reversible effects: 1) An increase in the number of errors: this effect was more pronounced on trials with long delay (8, 16 or 32 sec.) than on those with short delay (1 or 4 sec.). 2) An increase in reaction time on presentation of the sample color. 3) An increase in reaction time on presentation of the two colors for delayed matching to the sample color. The results indicate a participation of the inferotemporal cortex in short-term retention of visual stimuli. The effects of inferotemporal cooling on reaction time, especially choice reaction time, may be secondary to the difficulty that inferotemporal lesion induced in mnemonic function.

Supported by NSF grant BNS 76-16984.

2606 IDENTIFICATION OF RETINAL TERMINALS IN THE CAT SUPERIOR COLLICULUS: ELECTRON MICROSCOPIC AUTORADIOGRAPHY AND DEGENERATION. Mary Behan and John K. Harting. Univ. of Wisconsin, Madison, WI 53706.

We have used the EM-autoradiographic method to determine specific details regarding the terminations of retinocollicular axons in the cat. Our findings confirm and extend earlier studies which have shown that while the major portion of the contralateral projection terminates within the stratum griseum superficiale (SGS1 of Kanaseki and Sprague, '74), retinal efferents also end within the more ventrally located SGS2 and SGS3. With a data tablet digitizer interfaced with a computer, we measured the mean diameter of terminals in sublayers of the superficial grey. These data reveal that contralateral retinocollricular terminals can be grouped into two size classes. The larger terminals of the contralateral SGS3 (1.87 ± 0.72 μm) are smaller (1.40μm ± 0.33 μm) ending within SGS1, and the larger (1.75μm ± 0.67 μm) within SGS3. The mean size of terminals in SGS2 is intermediate between that of SGS1 and SGS3. Furthermore, we have found that the ipsilateral retinocollicular terminals, which end primarily in the upper 100μm of SGS2, correspond in size with the larger terminals of the contralateral SGS3 (1.87 ± 0.72 μm). Thus, it appears that there is a gradient of retinocollicular terminal size within the superficial grey: small terminals are more dorsally located and large terminals more ventrally located. This suggests a correlation with the distribution of W and Y cell axons as established by electrophysiological methods. While we have been able to identify different sized retinocollicular terminals, we have not been able to correlate this difference with either vesicle shape or type of synaptic density. Thus, retinal terminals contain round vesicles and form asymmetric contacts and the morphology of these terminals appears consistent in all layers of the superficial grey. However, there is a correlation between the morphology of profiles post synaptic to retinal terminals and depth: 40% of postsynaptic profiles in SGS1 (W cell input?) contain round vesicles in contrast to 23% in SGS2 and 19% in ipsilateral SGS2 (Y cell input?). Mean counts of degenerating terminals from 7 contiguous sample areas across the medio-lateral extent of the ipsilateral colliculus indicate that 5% of retinal terminals are from the ipsilateral eye. This figure of 5% is also obtained from counts of labelled terminals. However, presumably as a result of the pattern distribution of the ipsilateral projection, the proportion of ipsilateral terminals in small areas varies considerably.

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with data about the meaning of the stimuli in the current context, and at a still later time the same cells receive firings in visual cortex is related only to the stimulus; at a level in the late post-stimulus interval was recoded to a different activity had disappeared by this late epoch (M were assessed by calculating a linear regression line through a 250msec backward-averaged pre-response histogram and testing the metacontrast masking on the other side) than when he pressed the trials was greater when the monkey responded on the correct side for both M and B trials (M, t=0.49, N.S.; B, t=1.44, N.S.).

These results show that the first burst of stimulus-evoked firings in visual cortex is related only to the stimulus: at a later interval in the same cells this information is combined with data about the meaning of the stimuli in the current context, and at a still later time the same cells receive information about the organization of the behavioral response.
NEURONS IN THE NUCLEUS OF THE BASAL OPTIC ROOT (ACCESSORY OPTIC SYSTEM) OF BIRDS RESPOND PREFERENTIALLY TO VERTICAL STIMULUS MOVEMENT. Sheila Burtner* and Josh Hallman*, Dept. of Biology, City College of the City University of New York, New York, NY 10031.

The accessory optic system, which is found in all vertebrate classes, has been implicated in the visual control of eye and head movements. In birds, the accessory optic system contains the nucleus of the basal optic root (nBOR), which is also known as the ektomamillary nucleus and is homologous with the medial terminal nucleus of nmannia. The nBOR has several interesting anatomical features: (1) its principal input is from the displaced ganglion cells of the retina, a clearly identifiable collection of neurons, (2) it projects directly to the oculomotor complex, providing a short loop for visual control of eye movements and (3) it also projects to the vestibulo cerebellum both directly, as mossy fibers and indirectly, via the inferior olive, as climbing fibers.

Single unit recordings were made from nBOR in chickens. Preliminary results show that individual neurons have extremely large receptive fields and respond preferentially to stimuli moving vertically at slow velocities (about 1 deg/sec). They respond best to large stimuli and poorly, if at all, to small or stationary stimuli. Neurons with a preference for movement in the horizontal direction appear to be absent from this nucleus. The physiological properties of the neurons are, thus, strikingly consistent with the anatomical findings of Brecha and Easter (1973). The FEF neurons, which show that projections of nBOR in the oculomotor complex are to regions which control vertical eye movements. (Supported by NIH Grant EY-2937).

THE EFFECT OF INTEROCULAR ADAPTATION ON CONTRAST MODULATION, J. Canting*, I. Bodis-Wollner, and C.D. Hendley*, Mount Sinai School of Medicine, New York, NY 10029.

A contrast modulated grating is the sum of a steady and a counterphase flickering grating of the same spatial frequency. Adaptation to a steady grating suppresses two mechanisms subserving detection of this stimulus: as a result of adaptation, the threshold of the counterphase component becomes independent of the stationary contrast (Bodis-Wollner and Hendley, J. Physiol., 1979). In addition, adaptation "enhances" the sensitivity to the counterphase component above 10% mean contrast. This paradoxical result is presumably due to some sort of "adapting out" of the steady component which at high mean contrasts normally reduces sensitivity to the counterphase component. In the present study the effect of contralateral adaptation on contrast modulation sensitivity was explored for a 6 deg grating modulated at 8 Hz. Contrast modulation thresholds were established both before and after adaptation for mean contrasts ranging from detection threshold to 40%. There was little and often insignificant effect of adaptation on the modulation sensitivity near detection threshold, but in the range above 10% contrast we again found a paradoxical increase in sensitivity similar to the effect of monococular adaptation. These results indicate that the interaction of the stationary and counterphase components of the 6 deg grating are affected by interocular adaptation, and therefore the major site of this interaction between the stationary and counterphase components must be at or beyond the point of binocular convergence.

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THE MONKEY FRONTAL EYE FIELDS HAVE A NEURONAL SIGNAL THAT PRECEDES VISUALLY GUIDED SACCADES. M. C. Bushnell* and M. E. Goldberg, Lab. Sensorimotor Res., National Eye Institute, NIH, Bethesda, MD 20014; and Dept. Neurology, Georgetown Univ., Washington DC.

Electrode stimulation of the monkey frontal eye fields (FEF) elicits contralateral eye movements, and half of these movements are evoked by a stimulation that is not preceded by a visually guided saccade. This is in contrast to the situation in the cat, in which no visually guided saccades are evoked by FEF stimulation. The present study is aimed at determining whether or not FEF neurons show an enhanced response before visually guided saccades in the monkey.

The FEF of the macaque monkey was stimulated using a microelectrode at sites of visually responsive neurons saccades, thereby evoking a range of eye movements. Nearly half of the visually responsive neurons in the FEF showed more vigorous responses to the stimulus onsets when the monkey was preparing to make a saccade to the receptive field stimulus. This enhancement was specific to the saccadic motion component of the neuronal discharge, and was not seen when the monkey made a saccade outside the receptive field or when he touched the stimulus in the receptive field without making a saccade to it.

Supported by NIH Grants MH28649 and NS11677.

STIMULATION OF THE CAT SUPERIOR COLLICULUS EVOKES EYE MOVEMENTS SIMILAR TO those produced by visual stimuli. H. Peter Crampon and Barry E. Stein, Dept. Physiol., the Coll. of Va., Richmond, VA 23298.

The superior colliculus is believed to be involved in orienting receptor organs to a variety of sensory cues. Yet, while the influence of the colliculus on eye movements has been studied extensively, relatively little information is available about its control of ear movements. This information is important because the cat colliculus and, if so, what its relationship to the eye movement nasa. A cylindrical steel chamber was implanted over a cranial opening in 13 weeks prior to stimulating the cat's colliculus and, if so, what its relationship is to the eye movement nasa. A cylindrical steel chamber was implanted over a cranial opening in 13 weeks prior to stimulating the cat's colliculus and, if so, what its relationship is to the eye movement nasa. A cylindrical steel chamber was implanted over a cranial opening in 13 weeks prior to stimulating the cat's colliculus and, if so, what its relationship is to the eye movement nasa. 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A cylindrical steel chamber was implanted over a cranial opening in 13 weeks prior to stimulating the cat's colliculus and, if so, what its relationship is to the eye movement nasa.
2618 ELECTROCORTICAL RESPONSES TO VISUAL SPATIAL FREQUENCY IN BEHAVING MONKEYS. Richard Coppola and Richard K. Nakanuma, NIH, Bethesda MD 20010

Cortical potentials to visual stimulation by gratings of various spatial frequencies (SF) were obtained from alert rhesus monkeys to gain some insight into the processing of visual stimuli by pattern information. Stimuli consisted of vertically oriented sine wave or square wave gratings in the range 0.125 to 8 cycles/degree. The results were presented on a computer controlled CRT screen in both pattern appearance-disappearance and pattern reversal sequences. Contrast was held constant at 0.44 and luminance was constant at 0.2 ft-L. The animals had chronically implanted electrodes of stainless steel or platinum wire with ball tips placed on the dural surface at striate (S), prestriate (PS) and inferior temporal (IT) locations. The animals were trained to attend to the screen as follows. A trial was initiated by a lever press which caused the grating to appear-disappear or reverse in contrast each 250 msec. After random time periods the screen activity stopped and the animal had to release the lever to get a water reward. 500 msec from the start of the trial the amplified electrical activity from the electrodes was digitized with correct trials saved and averaged.

The appearance response at striate consisted of a positive component at 100 msec latency whose amplitude decreased with SF and a negative component at 150 msec which was relatively flat with SF. The prestriate response had three components: positive at 110 msec that peaked at low SF and then declined with increasing SF; negative at 130-150 msec that peaked with SF and; positive at 190-210 msec that increased slowly with SF. The IT response had a negative component at 100-120 msec and a positive at 190-210 msec both of which were relatively flat with SF. The largest difference between sine wave and square wave gratings was for the late positive component at PS and IT, which were smaller for the late negative component at PS and IT, which were smaller for the late positive component at PS and IT, which were smaller.

We have examined the responses of cortical units to monocular and binocular visual stimulation in cats in which the optic chiasm had been surgically-sectioned. The effect of this manipulation is to interrupt the direct retina-geniculate-cortex pathway from the contralateral eye, leaving the connections from the ipsilateral eye intact. Quantitative study of over 100 cortical units located near the border between areas 17 and 18 (with receptive fields located near the vertical meridian) showed that some units were driven exclusively by stimuli presented through the ipsilateral eye. However the firing rate of more than half of the units encountered could also be influenced by stimulation through the contralateral eye. The effect of visual stimuli presented through the contralateral eye could be excitatory or inhibitory.

We tested cortical units for their sensitivity to the retinal disparity of binocularly presented stimuli (corresponding to different positions in depth) and also for sensitivity to different directions of motion in three-dimensional space. While many units did not respond differentially to visual stimuli with varied locations or trajectories in depth, a subset of the units encountered were highly sensitive to the position and/or direction of movement of stimuli in 3 dimensional space.

Units showing strong binocular interactions were clustered within what appeared to be hemicylinders in the visual cortex. Within these hemicylinders which extended from the cortical surface to the geniculate-recipient zone in layer 4, successively-responded units were sensitive to particular characteristics of binocular stimuli. On a given penetration through the superficial cortical layers one might encounter cell after cell which responded optimally to stimuli moving toward the animal. Other penetrations yielded units which responded optimally to stimuli moving sideways at a given depth. Binocular interactions were most pronounced in units of the supragranular cortical layers and nearly absent in layer 4. In the infragranular layers the large-field complex cells were often binocularly-activated but displayed no selectivity for disparity. Some units in cortical layer 6 did however respond differentially to stimuli at different disparities.

The responses to stimulation of the contralateral eye were abolished or much reduced after inactivation of the visual cortex on the other side of the brain indicating that the major pathway mediating these responses is the corpus callosum.


Two groups of kittens were reared with a combination of monocular and directional deprivation. Group A (3 animals) had one eye sutured shut at 2 weeks of age, with each kitten placed in a striped drum rotating continually around it in one direction for one hour per day, otherwise remaining in the dark. Group B (5 animals) was reared similarly, except that the direction of drum rotation was reversed at 5 weeks of age. These rearing conditions were continued until 12 weeks of age, then the animals remained in the dark until electrophysiological recording. Cells recorded from visual cortex were classified as unidirectional, bidirectional, and omnidirectional or "hard to drive"; according to their ocular dominance; and various other characteristics.

The percentage of omnidirectional or nonspecific cells ranged from 22% to 41% for the deprived animals, compared to 6% to 15% for normal animals. Omnidirectional cells were found in substantial numbers in all layers of the cortex in the deprived animals, interspersed with specific (unidirectional or bidirectional) cells. Very few cells were driven by the closed eye; of those that were, most were nonspecific. The results of the animals in Group A showed that the monocular deprivation had a much more powerful effect than the directional deprivation for these rearing conditions. The results, when displayed in a 2 dimensional array with ocular dominance along one axis and directional sensitivity along the other, suggest that these cortical cells either lose their input from one eye, or lose their directional specificity, or both.


Following total removal of striate cortex, monkeys (and man) retain a large degree of complex visually-guided behavior. Yet no cortical area has yet been found whose visual responsiveness survives striate removal or inactivation. We report on a cortical area which does retain visual responsiveness after striate removal. This area, the Superior Temporal Polysensory Area, lies in the upper bank and fundus of the anterior and middle portions of the superior temporal sulcus. Neurons in this area respond to visual, auditory and somesthetic stimuli, and usually have receptive fields encompassing virtually the entire visual field (including both half-fields). About half the units are sensitive to the direction of movement of a stimulus, usually preferring movement in depth or movement towards the fovea from any point in the periphery (or vice-versa) (Neurosci. Abs. 3. 554).

In three monkeys, striate cortex was removed unilaterally. Several weeks later the monkeys were paralyzed, anesthetized with N2O, and simple unit responses were recorded from the Superior Temporal Polysensory Area ipsilateral to the lesion. Each animal was repeatedly recorded from. Over two-thirds of the units responded to visual stimuli in the half-field contralateral to the striate lesion. In this "cortically blind" half-field, receptive fields were still very large but there was little or no sensitivity to the direction of movement. Some of the units which had no direction specificity in the contralateral field did have some specificity in the ipsilateral field. Preliminary experiments in two monkeys indicate that the superior colliculus contributes to the visual responsiveness that remains after the striate lesion.
Without any further assumptions this measure was able to predict was underestimated, particularly at the low velocities. Thus the interactions corresponding to a given direction and speed. Properties. Stimulation of 2 positions in the same order as occurs to the 2 bars delivered separately. Both increases (facilitation) to light were flashed onto one or two of these regions. These results indicate that large numbers of medullary cells contribute to the movement responses recorded. (Supported by USPHS Research Grant EY 00008 from the National Eye Institute).

Many cells in the visual cortex respond much more vigorously to small moving objects than stationary ones. We have been examining the response properties of extrastriate pathways to also include more complex pattern discrimination. The extensive postoperative training needed for our animals, however, suggests that the geniculo-striate pathway is the primary pattern vision system in the normal monkey. (Supported by USPHS HL0756 & EY02941 and by Veterans Administration Research Funds)

We have been examining the response properties of extrastriate cells in the foveal projection pathway of alert, behaving macaque monkeys (Rhesus and Cynomolgus), and have identified substantial numbers of color-selective cells. Cells with a concentration of such cells in the anterior bank of the lunate sulcus, confirming Zeki’s (1973) earlier report of a color area (V4) in this region. Neurons are found in the inferior occipital sulcus. Electrolytic lesions along with receptive field properties permit us to distinguish color cells in the various subregions of extrastriate cortex. Our major concern has been to determine the color preference of extrastriate color cells. On the basis of cone inputs and spectral response peaks, there appear to be two distinct cell populations. The first group has opponent inputs from the long and middle wavelength cones (L/M cells) and excitatory/inhibitory peaks in the vicinity of 610-640nm (red) and 480-510nm (blue-green). The second group has synergistic input from long and short wavelength cones and antagonistic input from middle wavelength cones (S/LM cells). Our results verify that the de-striate monkey can discriminate partially-repolarized much more slowly. The depolarizations to movement were first summing the responses to the single bars, and then adding those interactions corresponding to the direction and speed. If movements stopped before reversals, the cells would partially-populate much more slowly. The depolarizations to movement were sometimes recorded, either with increasing speed of movement or when the movement was delivered to a cell, or in the small projection spot. A few, spontaneously-active spiking cells that were inhibited (hyperpolarized) by light were probably not fully OFF cells. Rather, these cells might have been included by other, more-ontip-excited cells, since one cell inhibited by light during movement resumed firing when movement was stopped.

Movement-sensitivities of visual interneurons in the medulla of flies. Robert D. DeVoe. Dept. Physiol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205. Intracellular recordings from the medulla (second optic ganglion) were made in intact, restrained flies that faced a hemispherical surface on which one or more lines and spots were projected from the rear. Most cells responded to light and movement with slow-wave depolarizations or hyperpolarizations; some had spikes in addition or alone. In the distal medulla, noisy hyperpolarizing responses to light along characteristic incoming axons of second-order, laminar cells. These cells responded with flicker-like oscillations to black-white alternations within moving gratings of 15°/cycle but not 2.5°/cycle. Medullary cells penetrated nearby responded to grating movements with similar but depolarizing oscillations that were 180° out of phase with the laminar cell responses and may have been driven by the laminar cells. These simple, oscillating responses to gratings are quite likely indicative of the inputs to the motion detectors. Most medullary cells had abrupt, maintained depolarizations in response to moving gratings. When the direction of movement was reversed, there were short, rapid, partial-repolarizations, but when movements stopped before reversals, the cells would partially-repolarize much more slowly. The depolarizations to movement were first summing the responses to the single bars, and then adding those interactions corresponding to the direction and speed. These results indicate that large numbers of medullary cells contribute to the movement responses recorded. (Supported by USPHS Research Grant EY 00008 from the National Eye Institute).

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INCREASE IN MONOCULAR DOMINANCE IN AREA 17 OF THE CAT FOLLOWING NEONATAL SECTION OF THE POSTERIOR CORPUS CALLOSUM. Andrea J. Elberger, Dept. of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104.

Neurons sensitive to binocular depth in areas 17 and 18 of the cat visual cortex. David Ferster* (SPONSOR: David H. Hubel). Dept. of Neurobiology, Harvard Medical School, Boston MA, 02115.

The landing reaction (LR) of flies: a model reaction for studying the neural basis of behaviour. Hendrik E. Eckert, Dept. Animal Physiol., RUB, P.O.Box 10248, D-4530 Bochum 1, W-GERMANY.

The landing reaction in flies of a simultaneous upwards throw of both forelegs begins an 'all-or-none' rule. Employing stripes moving apart and alternating, we estimated the LR - measured as the number of positive responses to 20 stimulus presentations - on the angular velocity, the extent and direction of stimulus motion, and the pattern motion. The LR was elicited only for different regions of the eye. (1) Despite the 'all-or-none' rule of the behavioural reaction, the neural circuit is activated in a graded manner: The LR, the extent of angular movement the higher the percentage of stimulus induced responses if immediacies from the posterior (or regressive motion) were stimulated; reversal of this direction of motion (progressive motion) exerts a graded inhibitory influence as can be shown by simultaneous and successive and progressive motion. (2) The most effective direction of stimulus correparation to be elicited for the LR for the response whose peak lies at 1.4 Hz. The position of these peaks cannot be altered by subsequent filtering stimulus. The mean time of response to pattern motion is the time-averaged outputs of the neural circuits. Thus separate neural pathways have to be postulated for mediating the influences. 

This behavioural investigation provides the means of studying the respective underlying neural circuits (e.g. depth perception, frontal sensory-motor control, sensory-motor detectors, excitation and inhibition). Paper: Eckert, H.; Fligge, B.; Hamdorf, K.: Naturwissenschaften, in press (1979).

Support: Grants Es 56/1a+b by the German Research Foundation (DFG) and grant BMS 74-21712 by the NSF.

Head tilt behavior during rearing in kittens whose visual cortical neurons have been modified by exposure to striped cylinders with the degree of modification of visual and somatosensory receptive fields during rearing on the modification of preferred orientation was observed between visual and somatosensory receptive fields from anterior to posterior in the tectum and the dorsal to ventral body axis represented from medial to lateral.

Normal visual responses, as reported by others, were recorded from the goldfish retina. The only visual responses recorded were somatosensory, although many fewer were found. The topography was similar to that of the cat fish but these neurons were really recorded outside the periventricular layer. In the goldfish a spatial correspondence was observed between visual and somatosensory receptive fields recorded from the same general location of the tectum. In mammals this correspondence is thought to facilitate visual orientation to non-somatic stimuli (e.g. Chalupa and Rhoades, J. Physiol., 210:393, 1977). The significance of the (apparently even more widespread) somatosensory representation in the blind fish optic tectum is under investigation.

Kittens were then prepared for neural recording using standard techniques of single unit recording. Bipolar stimulating electrodes were placed anteriorly to pass anteriorly through the retina and were advanced posteriorly to pass through the rostral to caudal body axis represented in the tectum and the dorsal to ventral body axis represented from medial to lateral.

Head tilt behavior during rearing in kittens whose visual cortical neurons have been modified by exposure to striped cylinders with the degree of modification of visual and somatosensory receptive fields during rearing on the modification of preferred orientation was observed between visual and somatosensory receptive fields recorded from the same general location of the tectum. In mammals this correspondence is thought to facilitate visual orientation to non-somatic stimuli (e.g. Chalupa and Rhoades, J. Physiol., 210:393, 1977). The significance of the (apparently even more widespread) somatosensory representation in the blind fish optic tectum is under investigation.

We now report the results of anatomical and electrophysiological experiments designed to determine if retinal afferents terminate in the retinal nuclei superior to the macula. Applications of 4% formaldehyde, 0.5% paraformaldehyde, 2.5% glutaraldehyde, and 1% osmium tetroxide revealed the presence of reaction product in the large "alpha" ganglion cells of the cat retina. The animals were permitted to survive for 24 hours before being sacrificed and perfused with a 2.5% glutaraldehyde, 0.5% paraformaldehyde, 2.5% glutaraldehyde mixture. The large ganglion cells in all retinae. Ganglion cells containing reaction product were found scattered in all retinal quadrants but appeared to cluster in an area encompassing the area centralis.

In an attempt to obtain confirmatory physiological data a series of animals were anesthetized and prepared for stimulation and unit recording. Bipolar stimulating electrodes were placed in the optic chiasm and superior colliculus and glass micropipettes were used for recording from cells in the nucleus centralis superior. Applications of 4% formaldehyde, 0.5% paraformaldehyde, and 1% osmium tetroxide revealed the presence of reaction product in the large "alpha" ganglion cells of the cat retina.


Prior work utilizing iontophoretic injections of horseradish peroxidase into the dorsal raphe nucleus revealed the presence of reaction product in the large "alpha" ganglion cells of the cat retina. The animals were permitted to survive for 24 hours before being sacrificed and perfused with a 2.5% glutaraldehyde, 0.5% paraformaldehyde, 2.5% glutaraldehyde mixture. The large ganglion cells in all retinae. Ganglion cells containing reaction product were found scattered in all retinal quadrants but appeared to cluster in an area encompassing the area centralis.

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Isolated perfused bulbog (Rana catesbeiana) retinas, treated with sodium aspartate, were studied to determine whether or not heavy metals affect photoreceptor function. Sodium aspartate was present in the perfusing solution to suppress the PII and the proximal PIII components of the electroretinogram and to isolate the distal PIII or late receptor potential. Retinas were first perfused for 30 or 60 minutes with control Tris-Ringer and then for an equal duration with Tris-Ringer containing the chloride salt of either divalent lead (Pb), mercury (Hg) or cadmium (Cd). The concentration range of the heavy metals was 1 to 50 μM. Following each experimental perfusion the retina was again perfused with the control Tris-Ringer solution so as to compensate for any normal decay with time and to examine the reversibility of the effects. Rod and cone responses to bright light flashes were separated using previously described techniques (Gillman, Vision Res. 13, 1021, 1974). Treatment of the retinas with either Pb, Hg or Cd resulted in decreases in the amplitude of the receptor potential of rods, but cones were never affected. The effects of Pb and Cd were reversible and concentration dependent. The effects of Hg were not reversible. On an equimolar basis Cd was two to three times more potent than Pb. Photoreceptor phototransduction exhibited a 0.7 log increase in absolute threshold. The results are consistent with reports describing acotopic vision deficits in human and non-human primates following exposure to either Pb or Hg. These observations suggest the involvement of rod photoreceptors as a primary lesion site in this deficit. The results predict that Cd exposure may produce clinical effects similar to those reported with Pb and Hg. (Supported by NIDBS HS0594 and PHS EY01839.)


The response of most photoreceptors to a brief flash of light consists of a slow graded depolarization or hyperpolarization. Although the early photochemical and biochemical stages of the process have been investigated in several vertebrate species, the mechanisms discovered so far can account for the time course of the response. The model originally proposed by Hodgkin and Fourquet to account for the dynamics of the vertebrate receptor has been applied to invertebrates. The model consists of a chain of exponential filters cascaded together. This model has been used with varying numbers of stages to describe the behaviour of several invertebrate and vertebrate photoreceptors.

We have shown previously that the frequency response function for light transduction is a useful method for testing the cascade model since the asymptotic gain and phase curves at high frequencies can be used to determine the number of stages involved. Unfortunately the signal to noise level of photoreceptor neurones always drops progressively with ascending frequency so that it is difficult to determine the validity of the model and the number of stages with accuracy.

In the current work we have used an averaging technique to improve the signal to noise ratio at higher frequencies. Photoreceptors in the compound eye of Rhodius prolatis were stimulated with repeated bursts of identical pseudo-random sequences of light flashes via a light emitting diode. The resultant membrane potential fluctuations were filtered and averaged. The averaged results were then averaged to reduce the inherent noise and the results processed as in normal linear systems analysis using a random impulse signal. This procedure resulted in improvement in the reliability of the frequency response function at higher frequencies, as measured by the coherence function. The frequency response functions for sequences were fitted by the normal form parameters of a polynomial into poles and zeros. All of the experimental results could be well fitted by a model which contained two second order poles and two first order poles. The presence of these first and second order poles indicates that there are temporal resonances occurring within the phototransduction mechanism and that the cascade model must be judged inadequate for the behaviour. In addition it was found that the phase portion of the frequency response function always lagged without limit at higher frequencies which were approached. This strongly suggests that there is a pure delay element of several milliseconds present in the phototransduction mechanism.


At least 25 neurons in the dorsal lateral geniculate nucleus of normal adult cats were classified both physiologically and morphologically. To do this, we used intracellular recording and iontophoretic injection of horseradish peroxidase (HRP). Y-cells, morphologically, are Guillery's class 1 cells, some are class 2, and the rest are intermediate between these morphological classes. Most X-cells, morphologically, are intermediate between Guillery's classes 2 and 3, while some are completely class 2 or 3.

Other morphological differences between X- and Y-cells were seen in our sample. 1) The X-cells have smaller somata. 2) X-cell dendrites tend to be elongated perpendicular to the lamina, while the Y-cells display no such asymmetry in their dendritic arborization. 3) Axons of most Y-cells issue collaterals within the region of the perigeniculate nuclear, while few X-cell axons have these collaterals. 4) Some X-cells issue intrageniculate axon collaterals, while none occur from Y-cell axons. 5) Dendrites of X-cells remain entirely within the lamina, while dendrites of Y-cells cross laminar boundaries.

In addition to the experiments on normal cats, preliminary data from monocularly deprived cats have been obtained. To date, only 7 cells from deprived animals have been analyzed. While some sizes appear altered by the deprivation, the normal structural/function relationship, which relates receptive field type to dendritic morphology, appears unchanged. More detailed morphological studies of geniculate X- and Y-cells in monocularly deprived cats are in progress.


Supported by N.I.H. grant EY01340 and N.S.F. grant BNS77-06785.


In the pigeon, single unit studies have shown that a majority of optic tectal neurones have receptive fields of relatively small moving stimuli. Most of these same neurons however, do not respond to large textured patterns and in many cases their receptive fields are completely inhibited by patterns moved 'in-phase' (same direction and velocity) with test stimuli (Frost, 1978). Therefore other than the fact that the response of the tectum is to 'notice' or respond to object motion and to ignore or veto self-induced motion produced by body, head or eye movements.

C-2-Dexoxyglucose (C-2-DG) autoradiography was used to reveal the relative responsiveness of tectal cells to small moving stimuli, to large textured patterns and to various combinations of these two types of stimuli. Lightly anaesthetized birds were injected with 50 μ Ci of C-2-DG via the brachial vein. They were then rapidly inserted into a stereotaxic instrument and one eye opened to view a tangent screen upon which stimuli were projected, while the other eye was closed and occluded. One group of pigeons viewed for 45 minutes a single spot of light (+1° diameter) as it was repeatedly swept forward along the horizontal meridian, while another group viewed the same stimulus patterned on the fovea. In other conditions birds viewed a large random dot pattern, with the same motion characteristics, and in-phase and anti-phase combinations of the test and background patterns. Autoradiographs were analysed using a computerized image processing system, so that the stimulated contralateral tectum could be compared to the ipsilateral tectum. The autoradiographs revealed no density increases in the region stimulated by the movement of the large random dot pattern with or without the moving test spot. In contrast, forward motion of the small test spot alone produced a clearly visible and discretely localized area of increased density extending through all tectal laminae. The line of increased density ran posterior to anterior along the tectum and was quite dorsally situated, thus suggesting that the lower visual field has a relatively larger tectal representation than the upper visual field.
DISFACILITATION AND INHIBITION IN THE VERTEBRATE RETINA.


The response properties of mudpuppy retinal neurons were examined by means of intracellular recording and current injection, and by studying receptive field properties. Most neurons with a predominantly depolarizing response show evidence of hyperpolarizing responses. For example, depolarizing bipolar cells of the mudpuppy exhibit a small hyperpolarizing prepotential (HPP) prior to depolarizing: in these cells, the HPP is more apparent with diffuse light stimulation than with a light flash restricted to the receptive field center. A similar HPP is also observed in the response of on-off amacrine and ganglion cells; hyperpolarizing current enhances the HPP while depolarizing current causes the HPP to diminish in amplitude. In contrast, the on and off IPSNs observed in on-off amacrine and ganglion cells reverse when small amounts (less than 0.1 μA) of negative current are injected. Of interest, different amounts of current must be injected to reverse the on and off IPSNs of some on-off ganglion cells. Finally, ganglion cells can be categorized on the basis of their hyperpolarizing response components. For example, our analysis indicates the existence of three classes of off-center ganglion cells. Sustained off cells, which display a maintained hyperpolarization during photic stimulation and a small depolarization at light offset, show few obvious signs of any inhibitory input. Two classes of transient off ganglion cells respond with a phasic hyperpolarization at light onset and a small hyperpolarization-hyperpolarization sequence at light offset: in transient type I cells, the initial negative deflection is enhanced by hyperpolarizing current while the off negative deflection is reversed by hyperpolarization and appears to be a true IPSN; in transient type II cells, both on and off hyperpolarizations reverse with negative current injection. These results are consistent with the prior suggestions by Frumkes and Miller that different latencies of neurons in the outer nuclear layer play a major role in information processing, and that several different classes of amacrine cells supply the inhibitory input to ganglion cells.

Supported by NIH grant ET-01802.

PROJECTIONS OF THE LATERAL SUPRASYLVIAN VISUAL AREA TO THE PONTINE NUCLEUS OF CATS.


The lateral suprasylvian visual area of cats receives a direct visual input from the dorsal lateral geniculate nucleus and contains several independent representations of the visual fields. The receptive field properties of cells in these lateral suprasylvian areas resemble those of pontine visual cells in that they are insensitive to the orientation of targets but responsive to their speed and direction. The present study was designed to determine: 1) Where in the pontine nuclei fibers from lateral suprasylvian areas terminate. 2) Which lateral suprasylvian areas resemble those of pontine visual cells in that they are insensitive to the orientation of targets but responsive to their speed and direction. The results of this study are consistent with the prior suggestions by Glickstein and Cohen that different latencies of neurons in the outer nuclear layer play a major role in information processing, and that several different classes of amacrine cells supply the inhibitory input to ganglion cells.

Supported by NIH grant ET-01802.

VISION

2641 VISION IN THE MACAQUE: VISUOTOPIE ORGANIZATION AND EXTENT.


The visuotopic organization of V2 was investigated in Macaques having two visual fields. Six monkeys were studied with multunit electrodes while immobilized and under N2O/O2 in repeated recording sessions. V2 surrounds striate cortex and resembles a virtually complete representation of the contralateral half field. It corresponds closely to von Bonin and Bailey's cytoarchitectonic area of V2. The visuotopic pattern is myelinated and cell-intriguing by a dense and homogeneous fiber pattern in layers IV to VI. Dorsolaterally, V2 includes most of the injection sites, and the posterior boundary of the occipito-temporal sulcus. It extends dorsomedially into the posterior bank of the parieto-occipital sulcus and ventromedially it includes the collateral sulcus, the posterior portion of the occipito-temporal sulcus and moves into inferior occipital sulcus. On the ventrolateral surface it includes the posterior bank of the inferior occipital sulcus.

The representation of the fovea in V2 is located ventrolaterally, adjacent to that of the foveal representation in V1. The representation of the vertical meridian in V2 is adjacent to that of V1 and the iso-eccentricity lines in V2 are continuous with those in V1. The representation of the horizontal meridian splits and forms the anterior border of V2, laterally, medially and in the calcarine sulcus. The lower visual field is represented dorsoventrally and the upper visual field is represented ventrally and ventromedially. Thus, the cat and owl monkey, V2 is a second order transformation of the visual field.

The research reported herein was supported in part by National Science Foundation Grant NSF-71-16894, "Visual Input to the Cerebellum."
A PRETECTAL PROJECTION TO THE DORSAL LATERAL GENICULATE COMPLEX

We here report that the pretectal region, like the superior colliculus, also sends a fiber projection to the dorsal lateral geniculate complex (LDg). While the tecto-geniculate projection is distributed mainly to the deep C-laminae, the pretectal projection projects mainly to the more dorsal laminae and to NIM. These observations confirm and extend earlier fiber degeneration findings described by Toth (1977).

Pretectal afferents are studied by autoradiography in 28 adult cats. Without exception, 8-amino acid injections centered in the nucleus of the optic tract (NOT) elicited labelling of the LDg complex on the ipsilateral side. In several of the animals, labelling was densest enough to be seen at low power with dark-field optics. In the laminar LDg, labelled fibres formed a rich network that was densest in layers A and A1 (including the monocular segment) and, variably, in layer C. It was not clear that any labelled fibres terminated in laminae C1-C3, for labelling in these layers was weak and due at least in part to perfusate fibres. Labelling of NIM was always as dense, and often denser, than that in the main LDg laminae. Extremely weak LDg labelling sometimes appeared contralaterally. It is striking that in the laminar LDg, the layers of densest labelling correspond to those richest in AcCh activity and that in the LDg complex as a whole, the labelled regions correspond to those known to receive retinal 1-cell input.

In cases of thalamic or tectal injection sparing the pretectum, such a pattern of LDg labelling was absent, though in the thalamic cases labelled fibres passed through LDg in straight-line trajectories and after tectal injections a terminal field appeared in C/C1. Pretectal deposits elicited labelling of LDg only when they involved NOT, save in one case (an olivary nucleus deposit with weak LDg labelling). Results in a case of BPF injection into LDg also suggest that the fibre projection arises in NOT, as most (but not all) BPF-positive cells in the pretectum were in NOT.

Since two amino acid deposits involving lateral NOT labelled mainly rostral LDg while 4 medial NOT injections labelled mainly caudal LDg, the pretecto-geniculate projection appears to have topographic order, at least along one main axis. Supported by NTR-1-R01-RE 0286-01 and NSF BNS 75-18758 & 78-10549.


At 10 days of age, the lateral rectus muscle was cut unilaterally in 6 kittens and bilaterally in one kitten. Experiments were conducted when the kittens were approximately 9 months old, and the properties of 468 ganglion cells were compared with the properties of 436 units recorded from normal cats.

The stimulus intensity required to elicit a firing rate of 200 spikes/sec was significantly higher for the units obtained from the experimental animals. This was found at all retinal loci tested. The maximum firing rate evoked by stimulating the receptive field center was significantly lower in the experimental animals at all retinal loci. The differences between the experimental and control animals were more marked for the units recorded in the area centrally. The correlation between the receptive field center size and conduction velocity found in normal animals was not found in the experimental animals.

These observations were made for units recorded from as well as from the unoperated eye.


Following an injection of tritiated proline into the dorsal part of the tuberal region of the hypothalamus, an extensive and dramatic pattern of transported protein is apparent within the ipsilateral superior colliculus. In one particular experiment the injection site of 8-proline lies primarily within the dorsal hypothalamic area (see figure). To a lesser degree the precursor spreads into the inferior hypothalamic area and the most medial locus tested. The maximum firing rate evoked by stimulating the receptive field center is significantly lower in the experimental cats than in normal cats.

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DEMONSTRATION OF OCULAR DOMINANCE COLUMNS IN NISSL STAINED SECTIONS OF MONKEY VISUAL CORTEX FOLLOWING ENucleATION. E. C. Haselkorn, T. J. Bellgowan, and V. A. Casagrande. Departments of Anatomy and Psychology, Vanderbilt University, Nashville, TN 37232.

Enucleation produces trophic effects in both the lateral geniculate nucleus (LGN) and visual cortex of monkeys. We studied the geniculostriate system of a monkey following long term monocular enucleation in order to determine 1) if deprived cells in the LGN matured, and 2) if any deprivation related changes can be demonstrated in visual cortex. The right eye of a wild adult cynomologus macaque was removed. Following a survival period of 29 months the remaining eye was injected with 500 µCi of 3H proline and both cortices were injected with 30% horseradish peroxidase (HRP). Two days later, the animal was perfused and appropriate sections were processed for autoradiography, reacted with diaminobenzidine, or stained with cresyl violet. Examination of the LGN showed that although cells in the denervated laminae were 39% to 53% smaller in area than their non-deprived counterparts, the percentage (90%-95%) of HRP labelled cells from the cortical injections was approximately equal in both hemispheres. This demonstrates that the deafferented neurons maintain cortical connections.

The right eye of a wild adult cynomologus macaque was removed. deprivation related changes can be demonstrated in visual cortex.

In Nissl sections, layer IV of striate cortex in both hemispheres showed alternating light and dark variations in staining that were comparable in width (300-500µ) to ocular dominance columns typically described in this species (Hubel et al. '68, '76, '77). Although adjacent light and dark bands were of equal widths, there were 20%-30% more neurons in the dark bands, and cells in these bands were 7%-14% smaller than those in light bands. Because of differences in the degree of overlap in the bands, we were unable to determine if individual cells in the darker bands were smaller. Comparison of the two hemispheres in the monocular region of cortex indicated that the darker columns represent the enucleated eye. With regard to these results, it is noted that a decrease in area and an increase in cell packing density has also been observed in cats following early nerve crush (Cragg, '75). Taken together, these results suggest that the introduction to transneuronal changes in the LGN, removal of one eye of a primate can result in dramatic changes in the visual cortex, even after maturity.

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LAMINAR ORIGINS OF IPSILATERAL TECTOBULBAR PATHWAYS. Virginia Holcombe and William C. Hall. Departments of Anatomy and Psychology, Duke University, Durham, N.C.

As part of an ongoing investigation of the laminar organization of the superior colliculus in the grey squirrel, Sciurus carolinensis, the ipsilateral pathways from the superior colliculus to the brainstem have been studied with the following anterograde and retrograde techniques. First, lesions of the directed leucine and/or lysine were injected electrophoretically into the superior colliculus 45-50 hours before sacrifice. Series of sections were dehydrated and exposed to photosensitive emulsion for 4-5 weeks, developed, and counterstained. HRP (30% in saline) was injected electrophoretically into area 24-44 24-48 hours before sacrifice; series of 40-48µm sections were processed with 3,3',5',5'-tetramethyl benzidine and counted for light substance. When tritiated amino acids are injected into the superior colliculus, a prominent well-defined efferent pathway can be traced laterally and ventrally through the ipsilateral brainstem. The terminal fields of this pathway include the lateral tegmentum and the dorsal lateral pontine nucleus.

When HRP is injected into the dorsolateral pontine nucleus and dorsally adjacent tegmentum, peroxidase-positive fibers were observed in the superior colliculus with the exception of stratum zonale. In a typical case, the majority of labeled cells are located in stratum griseum superficiale while the remaining cells are distributed throughout stratum opticum, stratum griseum intermediale, and stratum griseum profundum. In stratum griseum superficiale, the number of labeled cells increases caudally.

Injections of tritiated amino acids, as well as labeled leucine and lysine, into the superior colliculus superficialis suggest that the tectopontine projection arises in large part from superficial collicular laminae. Thus, the efferent projection from the entire colliculus, such as labeled following our HRP injection, can be the source of the projection to lateral tegmentum. We are currently seeking a more refined distinction between the cells of origin of these two projections by making a series of more restricted injections of HRP.

(Supported by NIH NS-09623 and NIH RSDA NS-25734.)

We have used an extensive use of the biotin-antibiotag autoradiographic tracing method in order to analyze the organization of retinocollicular pathways in thirty different mammals. Representative features of the following orders were studied: monotremes (Echidna, Ornithorhynchus); marsupials (Diprotodon, Tasmanian devil); and placental mammals (horse, pig, rabbit, baboon, cat).

The retinotopic organization of the contralateral projection is revealed anatomically in the optic tectum (colliculus). The ipsilateral projection is also restricted in its representation of the peripheral visual field, being densest within the medial portions of the stratum zonale and the region of densest contralateral input and always ends in a puffy shrew.

The ipsilateral retinocollicular pathway is less extensive than the contralateral in its overall projection (i.e., rostrally caudally and mediolaterally). In some of the lower forms this pathway is reduced, consisting of only one or two small patches of cells. In higher forms, the entire cortex is involved in the ipsilateral pathway. The ipsilateral projection is also restricted in its laminar distribution, usually being confined to one of the sub-layers of the superficial grey. In this regard, the Ipsilateral input present in lower forms terminates within regions of the stratum griseum superficiale. In higher forms, however, the ipsilateral projection extends ventrally through the layer I laminae into the layer II laminae. In this regard, the ipsilateral projection extends ventrally into the layer I laminae into the layer II laminae. The retinotopic organization of the retinocollicular pathways in thirty different mammals. Representation across mammals. In several cases, exceptions have been noted in order to generalize.

In all mammals thus far studied, retinal axons end exclusively within tectal laminae lying dorsal to and including the stratum opticum. The contralateral projection extends throughout the zones of the dorsal-caudal quadrant of the colliculus contralateral. The axons are densest within the medial portions of the stratum zonale and the dorsal part of the stratum griseum superficiale. Caudal zones of the contralateral projection are label than dorsal levels. To a slight degree in carnivores and in all primates the rostral-caudal extent of the colliculus.

Collicular pathways in thirty different mammals. Representation across mammals. In several cases, exceptions have been noted in order to generalize.

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In view of the large magnification factor associated with the contralateral representation of the visual field in mammals, it was hypothesized that the central representation of the spatial relationships between figural elements in these patterns would be most precisely elaborated within these areas. Accordingly, we examined the anterior or posterior regions of the visual field for the formation of a linear gestalt in cats and in several marsupials including the prosimian species. To a slight degree in carnivores and in all primates the rostral-caudal extent of the colliculus. Terminations are most dense within the dorsal part of the stratum griseum superficiale. In this regard, the ipsilateral projections end within the layer II laminae.

The results indicate that the formation of a linear gestalt in cats is immediate and unlearned, as judged by their near-perfect transfer of learned discriminations of obliquely oriented bars. In several cases, exceptions have been noted in order to generalize.

In contrast to our expectations, removal of area 17 and 18 had little effect on the grouping process, even under conditions of weak proximal stimulation. Moreover, direct stimulation of the mesially distributed lizards with similarly oriented line segments, a problem which requires a fine grain analysis of the geometry of the pattern elements, reveals that some of these cortices are important for more detailed spatial analysis, an interpretation that is consistent with parallel processing models of visual perception.

Supported by grants EYO577 and EYO1302.


The striate cortex of the tree shrew (Tupaia glis) does not have ocular dominance-columns but it does have a highly organized orientation column system. The majority of cells in all cortical layers are binocular and/or orientation selective. As part of an investigation into the anatomical organization of layer IV in the cortex, we have recently examined the distribution of synapses in layer IV, the site of major input from area 17 from the dorsal lateral geniculate nucleus (dLGN). Examination of the distribution of thalamic afferents to layer IV, and of the cell types within the layer, their dendritic spread and the axon trajectories of the output of the layer to the thalamus helped us to conclude that layer IV of the tree shrew striate cortex consists of two laminar sub-layers.

Axons from striate cortex were labeled using the Golgi-Restricted fragment of horseradish peroxidase (HRP) following injection of enzyme into the optic tracts. Axons from the dLGN previously shown to terminate in layers I, IIb and IV in tree shrew striate cortex (Harting, Diamond & Hall, JCN, 150, 73). We examined HRP-filled axons terminating in the dLGN laminae in the dorsal binocular region of area 17. Within layer IV, axons were seen to terminate only in the upper or lower half of the layer, with little encroachment upon the other half. The majority of the axons occupied the upper or upper half of the complex or lower half of the layer IV sub-layers, still spiny stellate, and pyramidal cells. Only stellate cells were present in the upper half of the layer; the lower half contained all three cell types. The dendritic fields of some of these cells extended into the upper and lower boundaries of the afferent laminae, still spiny stellate cells more centrally located in each sub-lamination having more spreading dendrites that occupied a greater thickness of the sub-lamination. The pyramidal cell soma and basal dendrites partially clustered at the upper border of the lower sub-lamination of layer IV. The apical dendrites of the pyramidal cells arborized in layer 11b. As such, the pyramidal cell cortex and the axon collaterals of the spiny stellate cells of the lower division. In contrast, the collaterals of spiny stellate cells in the superficial part of layer IV arborized in layer 11c.

Supported by NIH grants EY-01086 and EY-07013.


Using a two-choice discrimination apparatus, cats were tested for their capacity to polarize rectilinear arrays of pattern elements (dots or oriented line segments) into "rows" which appeared either vertically or horizontally oriented. The association between figural elements as "perceptual grouping", and in this case, the association between elements is governed by the relative proximity of one set of stimulus elements. In this regard, the association between elements is governed by the relative proximity of one set of stimulus elements. A set of lattice-like arrays was generated through systematic variation of the spatial relationships between the pattern elements. The results indicate that the formation of a linear gestalt in cats is immediate and unlearned, as judged by their near-perfect transfer of learned discriminations of obliquely oriented bars. In several cases, exceptions have been noted in order to generalize.

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EFFECT OF PRETECTUM ABLATION ON DETECTION OF BARRIERS AND APERTURES BY FROGS. David Ingle, Brandeis University, Waltham, Massachusetts 02154.

Earlier studies in our lab demonstrated that ablation of optic tectum in frogs had no effect on the ability to negotiate barriers or apertures. Our recording studies indicate that the cell bodies of the visual axons (16° of disparity between the two eyes), the distribution of interocular differences (IOD) in visual cortical cells' preferred stimulus orientations was centered about the rotation experienced during early development.

The present study tested the hypothesis that barrier detection critically depends upon the integrity of the posterior thalamus or pretectal region. Eight frogs with ablation of the posterior third of the thalamus were severely or totally deficient in turning beyond the terminal edge of a hemicylindrical barrier. Control lesions in anterior or middle thalamus were severely affected avoidance jumps elicited by visual threat.

Four animals which jumped randomly to the large barrier could rather consistently avoid a small (30° wide barrier) placed in a homogeneous white environment. The frogs, however, have avoided a white or black aperture in a small compartment instead of jumping through to escape noxious stimuli. We conclude that a spatial memory code to the other hemisphere, which is the function of the "small barrier" test can still allow looking discs - an ability associated with the optic tectum.

Because tectum ablation in the tree-shrew can also dissociate deficits in food-deprivation from habituation to raise the possibility that mammalian preptectum modulates comparable "locomotor orientation" by mammals.


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Previously (Shinkman, Bruce, & Isley, 1977) we showed that when kittens' early visual experience consisted of left- and right-eye visual fields of rotation in opposite directions, the visual axes (16° of disparity between the two eyes), the distribution of interocular differences (IOD) in visual cortical cells' preferred stimulus orientations was centered about the rotation experienced during early development.

The purpose of the present experiment was to assess the permanence of this effect, and its susceptibility to modification by subsequent normal visual experience during adulthood. Two kittens were reared in darkness except for two hours every day between the ages of 6 and 13 weeks. The kittens were visualized through goggles fitted with small prisms arranged so as to introduce a relative rotation between the visual fields in the left and right eyes. 8° counter clockwise in the left eye and 8° clockwise in the right eye.

Both kittens showed good visual behavior; accurate orienting toward and pursuit of visual targets, good discrimination on a visual cliff, and so forth. Subsequent- ly, the receptive field organization of visual cortex was studied, and the earlier results were confirmed. Except for the 100 degree distribution which was centered about 10° in the left eye and 20° in the right eye, the mean differed significantly from the expected value of 0° found in normally reared kittens.

The kittens were subsequently returned to the main colony without goggles where they experienced a normal visual environment for about 5 months. The kittens were then tested in the "small barrier" test and found in normally reared kittens, in terms of ocular dominance, orderly arrangements of orientation columns, and topographic representation of the visual fields. 8° and 10° barriers were centered about the expected rotation (X=19.7°), and the mean differed significantly from the expected value of 0° found in normally reared kittens.

Supported by NIH grants NB-17570 to P.G.S. and HD-03110 to the Biological Sciences Research Center.
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decay time of ~ 2.5 sec. These three events can be modelled by
liberate much of the K recorded in the proximal retina. On/off
K-increase was as fast as that of glial responses, so, on fortu­
gestig dead space may normally degrade the recorded K-response.
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recorded in the mudpuppy eyecup. A drop of Ringers was placed in
small, but on two occasions it was greater than 0.6 mM. When ob­
the eye to retard drying.

cells) and of Müller (glial) cells, and changes in [K+]0, were
postnatal, but is not a prominent feature until four weeks. The
time of birth, but at present there is little information available concerning the developmental timetable for retino­
geniculate synapse formation.

We have therefore used the electron microscope to study this problem in a series of cats ranging in age from newborn to adult (intermediate ages, 1, 2, 4, and 8 weeks). The animals were perfused with a mixture of paraformaldehyde and glutaraldehyde, and parasagittal slabs of the entire LGN were embedded in epon­
araldite. Photographic montages were constructed from thin sections restricted to lamina A near the center of the LGN; and terminals from the retina were identified on the basis of their relatively large size, round vesicle shape and pale mitochondria (RLP classification of Guillery, '69).

In the newborn, RLP terminals make simple axodendritic synaptic contacts. The postsynaptic profiles do not contain vesicles, nor do they contain fine filaments. The axodendritic terminals are located in the surrounding neuropil by glial sheets. At one week, RLP terminals are somewhat more evident than in the newborn kitten, but synaptic organization remains essentially unchanged. The encasement of synaptic zones begins at around two weeks postnatal, but is not a prominent feature until four weeks. The end of the first week also marks the onset of synaptic elements that contain synaptic vesicles. By eight weeks, synaptic complexity similar in configuration to those in the adult are common.

These results demonstrate that the morphological substrate for the display of information from the LGN to the thalamus at birth is present. The development of mature retino­
genicate synapses complexes does not take place until the second postnatal month, which may be related to the relatively late emergence of V-cell responses in the LGN (Daniels et al., '78).

Supported by NIH Grant EY01311.

RELATIONSHIP OF NEURONAL K-RESPONSES IN MUDPUPPY RETINA.

Chester Karwoski, Hiroshi Shimazaki* and Luis Proenza, Depts. of Psychology and Zoology, Univ. Georgia, Athens, GA 30602.

Intracellular responses of on/off-neurons (probably amacrine cells) and of Müller (glial) cells, and changes in [K+]0, were recorded in the mudpuppy eyecup. A drop of Ringers was placed in the eye to retard drying.

Within a thin layer of the distal retina, an apparent light-evoked K-increase was observed in about 50% of penetrations. The response was usually labile and its amplitude was typically small, but on two occasions it was greater than 0.6 mM. When ob­
erved, the distal K-increase always occurred with a latency, but additional data on its behavior remain limited. In the proximal retina, the light-evoked increase in K was quantitatively compared to Müller and glial responses. This K-increase had maximum amplitudes of up to 0.6-1.0 mM, which should evoke glial responses of 5-9 mV. The rise time and latency of the K-increase were generally slower than glial responses, sug­
gesting dead space may normally degrade the recorded K-response. In a few penetrations, however, the time course of the proximal K-increase was as fast as that of glial responses, so, on fortu­
tous occasions, dead space effects seem negligible. In these cases the half-decay time of K and glial responses was similar: 2-3 sec.

Müller cell responses show surround antagonism and decreased amplitude to high intensity stimuli, as previously shown in eye­
cups drained of vitreous. Müller cells in undrained eyes had large, stable resting potentials, but responses were small, aver­
aging only 1-3 mV at maximum.

All on/off-neuron responses show initial transient and later sustained components. The sustained component shows surround antagonism and decreased amplitude to high intensity stimuli, which together with other evidence suggests that on/off neurons liberate such much of their transmitter as is required to reach a constant level. Müller cell responses and recorded K may be related as follows: (1) on/off neurons release K in proportion to their level of depolarization, so that the K-increase in glial cells is directly related to the K-release in Müller cells; (2) the K-increase in glial cells is cleared by processes that have a half­
decay time of ~2.5 sec. These three events can be modelled by
passing on/off responses through a low-pass filter network hav­
ing appropriate rise and half-decay times. "Smoothed" neural responses bear striking similarities to K and Müller cell responses in all aspects yet examined.

Supported by NIH grant ET-00973 to L. M. P.)
VISION

2664 A SPECTRAL OPPONENT MECHANISM ENCODED BY ON-TYPE GANGLION CELLS OF RANA PIPiens. Karl Kicliter and Yuan B. Chen*, Laboratory of Neurobiology, University of Illinois at Chicago, Chicago, IL 60609.

We have previously shown that the major spectral opponent process on which the blue preference behavior of Rana pipiens is dependent, involves an interaction between the max3432 pm pigment of the green rod and the max2580 pm pigment of the principal and/or single cones (Kay and Kicliter, ARVO, 1978). We show here that some ON-type ganglion cells terminating in the anterior thalamus of these frogs encode the same spectral opponent process. Recordings were made from the anterior thalamus of Rana pipiens in or near the neuropils of Bellonci and the corpus geniculatum thalami, using tungsten-in-glass electrodes. Single ON-type units were isolated and were stimulated in the following manner. First, the receptive field of the unit was mapped. Then a spot of light was positioned within the receptive field center. The spot was composed of beams from 2 sources combined with a beam splitter. Beam 1 was composed of short wavelength monochromatic light (dominant wavelength 430 nm). Beam 2 could be formed of any of 10 monochromatic stimuli varying in wavelength from 430-650 nm; these 10 stimuli were adjusted for equal quantal flux with a neutral density wedge. One sec stimuli were presented and total spikes in a 2 sec interval beginning with stimulus onset were counted. Activity produced by stimulation with beam 1 alone is defined as baseline activity. When beams 1 and 2 were combined, the following results were obtained. When the dominant wavelength of beam 2 was less than 500 nm activity was increased over the baseline rate. When the dominant wavelength of beam 2 was 500-640 nm activity was reduced as compared to baseline. Not all ON-type units appeared to respond in this manner, but for some units encode the same spectral opponent process on which the behavior is based. This was not unexpected given the evidence that the blue preference behavior of Rana pipiens is dependent on information transmitted to the anterior thalamus by ON-type ganglion cells. Supported by NIH grants EY-02500 to E.K. and EY-01444 to Y.M.C.

2665 RETINAL INVOLVEMENT IN THE ELONGATION OF NEONATALLY SUTURED CAT EYES. Albert K. Kriby and Harold Weiss*. Ophthalmology Dept., Kreeger Eye Institute, Wayne State Univ. School of Medicine, Detroit, Mich. 48201.

Last year at this meeting we reported that the sutured eye of monocularly deprived (MD) neonatal cats and monkeys shows an increase in axial length compared to the fellow eye. In agreement with an earlier report by Wiesel & Raviola (1977) the deprived eye of the monkey was myopic and sometimes not. In the case of the non-myopic eyes the cornelan curvature usually compensates for the increase in axial length. The amount of myopia varies greatly from that predicted on the basis of axial length differences alone, and the cornelan changes, although in the right direction, are in most cases not sufficient to explain the absence of myopia in the non-myopic cats. Raviola & Wiesel (1978) further reported that dark rearing MD monkeys prevented the increase in globe length, seemingly implicating retinal absorption of light in the process. We sought to test this in cats by two methods: ablating all or part of the retina with laser photoablation, thereby destroying the involved retinal region and presumably preventing axial elongation; and by dark rearing MD cats.

(Submitted by B. K. is an Evelyn Sharp Fellow.)

2666 BINOCULAR COMPETITION: ITS ENHANCEMENT BY NOREPINEPHRINE. Baruch Kuppermann* and Takuji Kasamatsu. Division of Biology, Illinois College of Optometry, Chicago, IL 60616.

While dark reared MD cats show equal axial dimensions in both eyes, their MD litter mates, reared under normal lighting conditions, show increased axial dimensions in the sutured eye. Unfortunately we do not know the mechanism of axial elongation in MD cats or the compensatory mechanisms in the increase in axial length. It does, however, appear that some retinal absorption of light is required to provide visual feedback which somehow controls eye growth. Wiesel, T.N. & Raviola, E. 1977. Nature 266:66-68.


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2667 EYEBALL LENGTH CHANGES FOLLOWING EXPERIMENTAL SUTURING. Earl Kicliter and Yuzo M. Chino*, Laboratory of Neurobiology, University of Illinois at San Juan, CA 92939.

We have previously shown that the major sutured eye effect involves axial elongation which is dependent, involves an interaction between the max3432 pm pigment of the green rod and the max2580 pm pigment of the principal and/or single cones (Kay and Kicliter, ARVO, 1978). We show here that some ON-type ganglion cells terminating in the anterior thalamus of these frogs encode the same spectral opponent process. Recordings were made from the anterior thalamus of Rana pipiens in or near the neuropils of Bellonci and the corpus geniculatum thalami, using tungsten-in-glass electrodes. Single ON-type units were isolated and were stimulated in the following manner. First, the receptive field of the unit was mapped. Then a spot of light was positioned within the receptive field center. The spot was composed of beams from 2 sources combined with a beam splitter. Beam 1 was composed of short wavelength monochromatic light (dominant wavelength 430 nm). Beam 2 could be formed of any of 10 monochromatic stimuli varying in wavelength from 430-650 nm; these 10 stimuli were adjusted for equal quantal flux with a neutral density wedge. One sec stimuli were presented and total spikes in a 2 sec interval beginning with stimulus onset were counted. Activity produced by stimulation with beam 1 alone is defined as baseline activity. When beams 1 and 2 were combined, the following results were obtained. When the dominant wavelength of beam 2 was less than 500 nm activity was increased over the baseline rate. When the dominant wavelength of beam 2 was 500-640 nm activity was reduced as compared to baseline. Not all ON-type units appeared to respond in this manner, but for some units encode the same spectral opponent process on which the behavior is based. This was not unexpected given the evidence that the blue preference behavior of Rana pipiens is dependent on information transmitted to the anterior thalamus by ON-type ganglion cells. Supported by NIH grants EY-02500 to E.K. and EY-01444 to Y.M.C.

2668 BINOCULAR COMPETITION: ITS ENHANCEMENT BY NOREPINEPHRINE. Baruch Kuppermann* and Takuji Kasamatsu. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

It has previously been proposed that noradrenaline (NE) plays a role in synaptic plasticity in the cat visual cortex Naka, Takeda and Pettigrew, Science 204, 1976; Pettigrew and Kasamatsu, Nature 271, 1978). In the present study, cats given continuous local perfusion of 50 µM NE for seven days with a binocular environment were found to have a conspicuous lack of cells dominated by the ipsilateral eye, even though the ratio of binocularly:monocularity driven cells remained unchanged. This effect was not seen with the contralateral eye. The cells were of a variety of sizes but tended to be small, with a mean cross-sectional area of 33.4 µm² ± 15.2 µm² S.D.. Ipsilateral neurons were also found bilaterally in the parabigeminal nuclei, the corpus geniculatum thalami, using tungsten-in-glass electrodes. Single ON-type units were isolated and were stimulated in the following manner. First, the receptive field of the unit was mapped. Then a spot of light was positioned within the receptive field center. The spot was composed of beams from 2 sources combined with a beam splitter. Beam 1 was composed of short wavelength monochromatic light (dominant wavelength 430 nm). Beam 2 could be formed of any of 10 monochromatic stimuli varying in wavelength from 430-650 nm; these 10 stimuli were adjusted for equal quantal flux with a neutral density wedge. One sec stimuli were presented and total spikes in a 2 sec interval beginning with stimulus onset were counted. Activity produced by stimulation with beam 1 alone is defined as baseline activity. When beams 1 and 2 were combined, the following results were obtained. When the dominant wavelength of beam 2 was less than 500 nm activity was increased over the baseline rate. When the dominant wavelength of beam 2 was 500-640 nm activity was reduced as compared to baseline. Not all ON-type units appeared to respond in this manner, but for some units encode the same spectral opponent process on which the behavior is based. This was not unexpected given the evidence that the blue preference behavior of Rana pipiens is dependent on information transmitted to the anterior thalamus by ON-type ganglion cells. Supported by NIH grants EY-02500 to E.K. and EY-01444 to Y.M.C.

2669 SUBCORTICAL PROJECTIONS TO DORSAL LGN. Thomas P. Langer* and Carol L. Colby* (SPON: J.G. Malpeli). Dept, of Psychology, MIT, Cambridge, MA 02139.

We have examined the anatomical properties of those neurons in the superficial gray of the cat superior colliculus (SC) which project to the dorsal lateral geniculate nucleus (dLGN). In particular we have looked at their size, shape and position in relation to the known afferents and efferents of the superficial gray. Small dense electrophoretic injections of HRP were made in the C laminae of dLGN. Sections were reacted with TMB which is essential for demonstrating the very small neurons in the dLGN projection.

After injections centered in the C laminae of dLGN, labelled neurons were found in a compact group of cells in the lateral portion of the ipsilateral SC. They were distributed throughout the depth of the zone of horizontal cells (0-250 µm below the colliculo-dorsal zone) and less commonly within the margin of the zone of vertical cells (occasionally as deep as 350 µm). This region of the SC is known to receive a heavy input from the contralateral retina, the nucleus of the optic tract (N0T) and from both parabigeminal nuclei (PB) in addition to a lighter input from areas 17, 18 and 19 in visual cortex. The HRP-labelled neurons were among the smallest encountered in the SC (mean cross-sectional area 33.4 µm² ± 15.2 µm² S.D.). There was little dendritic filling but the location, size, shape and morphological density of these few cells seem to be the specific granule cells found in this region in Golgi impregnated material.

The collicular projection to dLGN is but one part of a network of interconnections involving SC, PB, NOT and dLGN. Labelled neurons were also observed in the parabigeminal nuclei, the N0T and in the parabigeminal mesencephalic reticular formation along with the adjacent central gray ventral to the inferior colliculus. Ipsilateral neurons were more numerous in all these regions and the NOT group of labelled cells was almost entirely ipsilateral. The cells were of a variety of sizes but tended to be smaller than the average within each region. The labelled cells in PB were among the smallest neurons in that nucleus. The collicular neurons labelled by HRP injections of the C laminae of dLGN are a discrete, well characterized population distinct from populations which project to other visual structures implicating a tight retinotopic correspondence in this projection. Other subcortical projections to dorsal lateral geniculate nucleus appear to originate from equally distinct populations of cells. Supported by NIH 1 F32 EY05290-01, BNS 78-10549, NIH 1 T31 RD-7484.
was no relative attenuation for low spatial frequencies. The spatial contrast sensitivity func-
tions (CSFs), which plot the reciprocal of the contrast threshold as a function of spatial or temporal frequency, for a normally reared cat and two cats raised with monocular eyelid suture. Using an operant technique of conditioned suppression, we trained cats to suppress a licking response whenever a CRT-generated, counterphased, sine-wave grating replaced a homogeneous-field of equal space average luminance. Contrast thresholds were estimated during a testing session using a staircase procedure.

The spatial and temporal CSFs of the monocularly deprived cats were similar to those of the normal cat. At lower temporal frequencies (e.g., 1 Hz), the spatial CSF showed an attenuation in sensitivity for spatial frequencies lower and higher than the peak frequency (0.5 c/deg). At higher temporal frequencies (e.g., 25 Hz), the spatial CSF showed reduced sensitivity for all spatial frequencies; however, there was no relative attenuation for low spatial frequencies. The temporal CSFs, which were measured at the most sensitive spatial frequency, showed a peak at 3 to 5 Hz, and attenuation for lower and higher temporal frequencies.

We found that contrast sensitivity of the deprived eye was nearly 1.5 log units lower than that for the nondeprived eye for all spatial and temporal frequencies investigated. The magnitude of this sensitivity loss was similar for both monocularly deprived cats, and has remained unchanged over a 9-month period of testing. A similar shape of the spatial CSF did not change with temporal frequency.

These behavioral results indicate that the visual impairment of the deprived eye is not due to a smaller size of the visual field, but represents a severe amblyopia that covers the entire spatial frequency spectrum. Moreover, these data complement the physiological result that there is a functional reduction of Y-cells in the deprived geniculate laminae.

EVIDENCE FOR AN EXTRA-GENICULATE THALAMIC RELAY FROM THE RETINA TO CORTICAL AREAS 19 AND CLARE-BISHOP IN THE CAT. Audie G. Leventhal, Jeremy Keens* and Istvan Törk*. School of Anatomy, Univ. of N.S.W., Kensington, N.S.W., Australia.

A direct retino-pulvinar projection has been reported in several species, including the cat (cf. Herman and Jones, Brain Res. 134:237-248, 1977). We have investigated the afferent and efferent connections of the RRZ of newborn rhesus monkeys. We infused the RRZ of newborn rhesus monkeys (to the eye) with tritiated proline by 1) injecting tritiated proline into one eye and, in the same animal, injecting horseradish peroxidase (HRP) into the visual cortex, 2) removing one eye of adult cats, injecting HRP into their visual cortex and investigating terminal degeneration electrophoretically.

The results confirm a direct retino-pulvinar projection since 1) autoradiographic label was present in the RRZ after 72 hours survival, 2) degenerating terminals were found in the RRZ after monocular enucleation and 3) HRP injections into the RRZ labelled ganglion cells. We observed that cells of the RRZ project to cortical areas 19 and Clare-Bishop but not to areas 17 and 18.

We reason that the projection from the retinal ganglion cells to the RRZ is a direct one, because the ganglion cells of the RRZ are not connected to the retina via the lateral geniculate nucleus. Therefore, a direct projection from the retina is the only possible explanation for the projection of the RRZ to the visual cortex.
2674 EFFECTS OF LESIONS OF PARIETO-OCcipital ASSOCIATION CORTEX UPON PERFORMANCE OF OCULOMOTOR AND ATTENTION TASKS IN MONKEYS. James C. Lynch and Jack M. McLaren. Department of Physiology, Mayo Clinic and Foundation, Rochester, Minnesota 55901.

Parieto-occipital association cortex has been implicated in the functions of visual attention and the processing of visual information by both clinical and electrophysiological studies. In the present series of experiments, four monkeys were trained to visually discriminate a 0.1° target and to follow its movements across a 52° square screen at velocities of 6-30°/sec and also as it suddenly jumped 8-24°. Eye position was measured with chronic extracarotidatic (EOG) electrodes which were amplified and stored for later computer analysis. After several weeks of baseline data collection, each animal received either a unilateral or bilateral lesion of the thalamic parietal cortex. The lesions were centered about the ventral portion of the nucleus. A poor representation from the periphery may not be surprising for SL since this region projects to regions of the paxillar which project to parietal cortex. (Supported by NIH grant EY 2940.)


Intralaminar thalamic stimulation (ILM) of this cell population in the command of eye movements (EM). Previous results were obtained in encéphale isolé. The head was fixated; vertical and horizontal electrooculographic recordings were made with implanted Ag-AgCl electrodes. Electrical pulses were applied through stereotaxically driven tungsten microelectrodes and were monitored by a digital computer. Pulses of 0.1 msec duration at a 250/sec rate were systematically used. Train duration was varied from 50 to 500 msec and intensity from 10 to 500 µA. Current threshold was defined as that sufficient to evoke EMs in 50% of the trials. All elicited eye deviations were conjugate, contraversive, and dependent on the alertness state of the animal. Three kinds of EMs were distinguished: (1) Goal directed i.e., the direction and amplitude depended on the initial eye position. The goal was an area about 5 deg located at least 10 deg away from the center. The greater the distance between initial position and goal, the more likely was a threshold stimulus to be effective, the amplitude was larger, and the latency of EMs was about 35 msec.


Seven macaque monkeys received argon laser beam lesions of the retina. After a recovery interval, the retina was removed. Several weeks later an intravital intravenous injection of 3H-proline was given to the animal with the idea that the proline would be taken up by the surviving ganglion cells. Monkeys without lesions but with proline injection served as control. Autoradiographs were stained to provide information on the distribution of surviving ganglion cells. No evidence has been found of contralateral neglect of unilateral visual stimuli following lesion. One monkey was trained on a visual fixation task and showed no tendency to ignore contralateral stimuli in paired stimulus presentations following lesion.

These results support the proposal that parieto-occipital cortex plays an important role in the initiation and control of visually-evoked eye movements, and suggest that damage to this cortical region along with its contralateral neglect which has been reported to follow parieto-occipito-temporal lesions in monkeys. (Supported by a grant from the Mayo Foundation and NIH grants EY 2640 and S 801 RR 5530-14.)


The goldfish retina grows both by expansion and by the addition of new cells at its margins. As the fish lengthens from 70 to 200 mm, the size of its retinal surface increases, but the distribution of grains from individual cases suggested that the foveal representation of horizontal and vertical saccades is still in progress. If dendritic tree size increases during growth, then a poor representation from the periphery may not be surprising for SL. Since this region projects to regions of the paxillar which project to parietal cortex. (Supported by NIH grant EY 2940.)


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2678 INTRINSIC PROCESSING IN THE VISUAL CORTEX OF PRIMATES.
Department of Psychology, University of Oregon, Eugene, OR 97403.
The retina contains a multiplicity of retinotopically organized, often
sectioned from 1-2 mm anterior to the chiasm.

2677 MODULATION OF LGN CELL RESPONSIVITY BY VISUAL ACTIVATION OF THE CORTICOCENGULATE PATHWAY.
Dept. Psychol. Univ. of Oregon, Eugene, OR 97403.
Corticocengulate (CG) cells in layer VI of striate cortex have receptive fields similar to non-CG cells of the same layer.

2679 RETINOPTIC ORGANIZATION OF AXONS IN THE OPTIC NERVE AND TRACT OF NORMAL AND SIAMESE CATS.
The retinotopic organization of axons in the optic nerve, chiasm, and tract was studied in normal and Siamese cats. Previous work indicated that the retinotopic order in Layers II and III of Area 17, but similar results were obtained from slices from both the prelunate gyrus and the inferior parietal lobule.

2680 Development of Somatom S y naps e i n V isual C o r tex (A rea 1 7) of the M acaque M onkey. S. M ates* & J. S. L und , D ep t. of O phthal mo lo gy, U niv e rsity o f W ashington, Seattle, WA 98195.
As a continuation of previous studies on the development sequence of neurons in visual cortex (Area 17) of Macaque monkeys, we have been studying the formation of somatotopic contacts of identified cortical cell populations receiving thalamocortical axons.

2681 The multiplicity of retinotopically organized, often interconnected cortical areas in the primate brain raise the problem of analysing intrinsic properties apart from those resulting from inputs from extraocular sources. In the case of isolated preparations of cerebral cortex to facilitate the electrophysiological and pharmacological study of neuronal properties under more controlled conditions than are found in vivo.

To determine at a cellular level the nature of the intrinsic processing, we examined in vitro perfused thin slices (600 μm) of monkey visual cortex that were sectioned normal to the pial surface to retain functionally intact their columnar organization. The slices were mounted in a stage of the microscope permitting direct visual placement of electrodes. In addition, cell soma and their processes could be visualized with Nomarski optics viewed through a microscope after labelling with horseradish peroxidase conjugated with the fluorogen tetramethyl rhodamine isothiocyanate. A multipot perforant synapses and small axons were also found normal perfusate with perfusates containing various ions or pharmacological agents.

In particular, it was the initial segment of the corticocortical pathway formed by the pyramidal cells in Layers II and III of Area 17, but similar results were obtained from slices from both the prelunate gyrus and the inferior parietal lobule.

Previous work indicated that the pyramidal neurons in Layers II and III could be well activated by brief bipolar electrical stimulation in vivo, whereas stimulation in vitro. Their responses are reversibly abolished by low calcium perfusate or by atropine, but not by nicotinic antagonists, suggesting that the activation is mediated by cholinergic synapses of the muscarinic type. Low frequency stimulation reversibly depressed the response with exponential decay and recovery phases. The depression was not blocked by the nicotinic antagonist d-tubocurarine, but rather this drug released a long latency component.

These results suggest a basis for intracolumnar adaptation and pericolumnal inhibition. (Supported in part by NSF grant BNS 75-08437.)

Immediately behind the eye, degenerating axons produced by localizing lesions form a well-localized group in both normal and Siamese cats. However, many individual axons are scattered away from the main groupings, and a considerable number of normal axons are mixed among degenerating fibers. Normal axon segments could be followed through the whole thickness of the section.

In normal cats, this localized grouping is maintained in the optic tract, coarser fibers lying laterally. It is true for Siamese cats. In both, there is a segregation of fiber sizes in the tract, coarsest fibers lying lateral. It is also true for Siamese cats. In both, there is a segregation of fiber sizes in the tract, coarsest fibers lying laterally. It is also true for Siamese cats. In both, there is a segregation of fiber sizes in the tract, coarsest fibers lying laterally. It is also true for Siamese cats. In both, there is a segregation of fiber sizes in the tract, coarsest fibers lying laterally. It is also true for Siamese cats. In both, there is a segregation of fiber sizes in the tract, coarsest fibers lying laterally.
The terminals of retinal ganglion cell axons (ON) were examined in the optic tectum of R. pipiens by EM. ON were identified by four categories: (1) ONs were labeled with HRP injected into the optic tectum; (2) 3H-proline was injected intracranially; (3) ONs were labeled by 3H-proline injected into the optic tectum; (4) HRP was applied to the optic tectum or nerve fiber to OT diffusely. The last method labeled ON in large numbers, and its morphology was used for useful survival periods. For details of methodology and background see Scali and Colman, Br. J. 74, Colman et al., Br. J. 76, Magoun* J. L. Bixby* and D. C. Van Essen (SPON: M. Konishi). Maunsell* J. L. Bixby* and D. C. Van Essen (SPON: M. Konishi). Maunsell* J. L. Bixby* and D. C. Van Essen (SPON: M. Konishi).}

The relation between the borders of MT are not always horizontal. The complexities are indicated by several observations. The myeloarchitecture provides an adequate basis for identifying the layer A, whereas type 2 was prevalent there and was the most numerous throughout the superficial tectum (30 days after eye removal). Since OT labeled by HRP were present in layer A and maintained intact in layers A, C, and E in significant numbers for 30 days, it may be that some type 2 were OT. Few terminals having the characteristic of types 2 and 3 were observed in the present study, while the impression was gained that the population of terminals is more highly differentiated than a four category schema would suggest. (Supported by NSF grant BNS 75-31088.)

The topographic organization of MT in the macaque is similar to, but more complex than that found in the owl monkey and the bushbaby. The complexities are indicated by several observations. a) Receptive fields of cells encountered along the perimeter of MT are not always close to the perimodal of the visual hemifield. (vertical meridian and far periphery), and fields recorded midway between the borders of MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. b) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. c) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. d) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. e) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. f) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. g) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. h) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. i) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. j) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. k) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. l) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. m) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. n) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. o) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. p) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. q) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. r) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. s) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. t) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. u) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. v) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. w) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. x) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT. y) The relationship between the hubbub and MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field location sometimes occur over short distances within the same cell. z) Anatomical experiments demonstrate instances of single loci within stripe cortex projecting to multiple sites within MT.
CONNECTIONS OF THE CLARE-BISHOP AREA WITH AREA 17 AND THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT. A. J. McGrath, G. R. Leichnetz, and J. Astruc, Department of Anatomy, Medical College of Virginia, Richmond, Virginia 23298.

The Clare-Bishop (CB) area of the cat cortex bears the name of the investigators who, first (1954) recognized a region, located primarily on the lateral aspect of the middle suprasylvian gyrus, which electrophysiologically responded to both striate cortex and optic nerve stimulation. More recent studies (Palmer et al., 1978; Kennedy and Magin, 1977) have supported its role in visual and/or oculomotor functions. Anatomical studies were performed in order to provide a morphological basis for its proposed function. In the process of this investigation it was found that a substantial reciprocity of connections exists between the CB area and Area 17, using both orthograde (silver impregnation) and retrograde (HRP) methods. Silver techniques (Kouta, Fink-Helmer) revealed that CB projects primarily to laminae III-IV of Area 17. However, some degenerating fibers were also observed to traverse lamina II and enter lamina I, giving rise to preterminal and terminal degeneration running parallel to the surface of the lateral gyrus. HRP studies, on the other hand, revealed that medium-sized pyramids located primarily in lamina III of Area 17 projected to the CB cortex. Anterogradely transported HRP also confirmed the presence of a terminal field in both laminae III and I of Area 17. In addition to finding these intimate connections of CB with Area 17, it was also observed that the Clare-Bishop area apparently receives substantial input directly from the dorsal lateral geniculate nucleus (dLGN). Whereas earlier investigations (Bethin and Jones, 1971; Rosenquist et al., 1974) have held that CB only receives innervation from the medial interlaminar nucleus and laminae C of dLGN, this study demonstrated that all of the main laminae (A, Al and C) have direct projections to the CB cortex. Thus it appears, based on the data presented in this study, that the Clare-Bishop area is an integral part of the primary visual cortex in the cat, due to its receiving input directly from the dorsal lateral geniculate nucleus, and its heavy reciprocal connections with Area 17. The data from this study provide an important anatomical substratum for CB's proposed role in visual activities.


The spatial and temporal modulation transfer functions (MTFs) of two pigtailed macaques (Macaca nemestrina) were measured behaviorally before and after small laser burns were placed in their foveas. Fundus photographs confirmed the location, size and completeness of the lesions.

The post-operative spatial MTF revealed no loss of sensitivity at the lowest spatial frequencies, a loss of less than 0.5 log unit at middle frequencies and a loss approaching 1 log unit at the highest spatial frequencies. The temporal MTFs measured with the 5° stimulus showed no loss of modulation sensitivity. However, the temporal MTF for the 0.5° stimulus indicated a 0.3 log unit loss of modulation sensitivity at the low frequencies and no loss at the high temporal frequencies. These results will be discussed in relation to the spatio-temporal properties of human foveal vision.

Supported by Grants ES01885, and ES01248 from NIEHS and MH1752 from NIH. DOR Report No. UR-3490-1626.
Large evoked potentials are elicited in humans by dynamic random-dot correlograms of binocularly identical noise alternating with binocularly positively-correlated noise (Julesz, Kropfl and Petrig, 1978). These correlograms contain no monocular cues; thus the evoked potentials are an objective indicator of a cyclopean perception (Julesz, 1971). We tried to elicit evoked potentials to these stimuli in alert macaque monkey so as to compare them with human evoked potentials and to develop a fast, objective test for cyclopean perception in animals. In order to test silently a chair placed facing a rear-projection screen. Dynamic random-dot correlograms, alternating at 0.5 Hz, were generated at 60 frames/sec. Stimuli for the left and right eyes were red into the red and green channels, respectively, of an Advent projection TV set. Stereo separation was achieved by placing red and green filtered filters over the left and right eyes, respectively, were worn by monkey and human observers. After every eye reversal, left and right dynamic noise was identical giving the percept of a flat depth plane; after every odd reversal, dynamic noise for one eye was the negative image of the other for the other eye giving rise to binocular rivalry and an uncertain depth percept. To test for monocular cues, we used three control conditions: A) placing identically colored filters over both eyes; B) vertically shifting the green image relative to the red image; C) placing a 0.5 log neutral density filter in front of the green filter (this delays conduction from that eye (Julesz and White, 1969) thereby reducing the amount of time the two images are correlated). In a fourth control condition (D), 0.5 log neutral density filters were placed over both filters: this delayed conduction from one eye equally so that the images appear as in the cyclopean condition but dimmer. While the cyclopean condition and control D gave large evoked potentials, controls A and B gave a greatly decreased evoked potential. This proves that the evoked potential was a response to the cyclopean aspects of the stimulus. Human sub­jects were told to look at the screen’s center whereas the monkey gazed wandered freely over the entire screen. Nonetheless, the monkey’s evoked potentials were as large as those of the human, demon­strating that the method does not alter the nature of the stimulus. This result testifies to the robustness of evoked potentials to dynamic random-dot correlograms and extends their usefulness as a test for cyclopean perception. (Supported by NIH NS-12131, NSF BNS-1969-1500, the Spencer Foundation and the Sherman Fairchild Scholars Fund)
The present study compared pre and post EM lesion behavior of pigeons to optokinetic stimulation presented both binocularly and monocularly. Single EM cell response characteristics were also investigated.

Before EM lesions, binocular head nystagmus was normal and characterized by a pursuit phase where head velocity matched stimulus velocity, both for clockwise and counterclockwise motion. In contrast, monocular head nystagmus approximated binocular conditions only with forward motion (posterior to anterior in the visual field). Both binocular and monocular lesions were far less effective in producing nystagmus and when it occurred head velocity was inappropriate. After unilateral EM lesions, head nystagmus was not elicited by forward optokinetic stimulation of the contralateral eye. Post-lesion binocular nystagmus resembled pre-lesion monocular responses.

Electrophysiological recordings of EM cells showed they were directionally selective, and preferred slow velocities of stimulus movement. Large single spots of light were generally ineffective, whereas very large textured patterns were optimal. These cells also exhibited a high spontaneous firing rate which was modulated up and down for preferred and null directions of motion respectively.

These observations lend support to the notion that the accessory optic system involves in the processing of whole-field motion required for stabilization of the visual world.

The critical period in kitten visual cortex begins around the time of eye opening and ends at three months of age. We were interested in determining whether visual experience plays a role in the timing of the critical period or if it reflects a fixed maturationally programmed process. Kittens were deprived of visual input from birth to an age of 14-16 weeks by binocular lid suture or rearing in complete darkness. They were then given monocular experience for a period of up to 1 year.

Prolonged monocular experience following binocular lid suture resulted in little improvement of visual acuity for simple behavioral tasks (placing, tracking, avoidance, avoidance of a visual cliff). Recordings in area 17 revealed no dominance of the open eye. Twenty eight percent of the cells were driven only by the open eye, 27% only by the closed eye, and 21% were binocular. Twenty four percent of the cells were visually unresponsive. Many visually responsive cells, particularly binocular ones, had abnormal receptive fields. There were no significant differences in cell sizes between LGN laminae associated with the closed eye. These cells typically had abnormal receptive fields. Fourteen percent of the cells were binocular, and 6% were driven only by the closed eye. These cells typically had abnormal receptive fields. Anatomical studies of these kittens are in progress, but preliminary results suggest that only slight shrinkage (10%) occurs in geniculate laminae associated with the closed eye.

We conclude that in the total absence of visual stimulation considerable plasticity is maintained in visual cortex. Apparently there is sufficient visual stimulation through closed lids to produce permanent abnormal development.
CHRONIC BLINDNESS FOLLOWING NONVISUAL CORTICAL LESIONS IN MONKEYS. Richard K. Nakamura and Mortimer Mishkin, Lab. of Neuropsychology, NHM, Bethesda, MD 20892.

We previously reported that blindness can be produced in monkeys by a large cortical injury that preserves all areas necessary for visual discrimination learning (Neurosci. Abs. 3:571, 1977). The lesions spared visual cortex (striate, prestriate, and inferior temporal) and limbic cortex (medial temporal, ventral frontal, and cingulate) but included all other cortical areas. A normal paralysis of the eye and the lesion in one hemisphere, the other hemisphere having been left intact but visually desensitized by optic tract section and forebrain commissure ligation. Intracerebral recordings in this way were behaviorally blind for periods ranging from 25 to more than 400 days, despite anatomical and physiological evidence that the retino-geniculo-striate pathway in the hemisphere with the ablation was preserved as intended.

The extreme variability in the duration of the blindness has not merely been puzzling but has impeded analysis of the phenomenon. New studies indicate that this variability can be eliminated and a predictably long-term effect produced in two different preparations.

First, the original findings suggested a species difference that has now been confirmed with additional animals given the standard lesion. The larger monkey species (Macaca mulata) have recovered from blindness after a median of only 28 days, none of four cynomolgus monkeys (Macaca fascicularis) has been shown to recover despite a median postoperative survival period of 180 days. Second, the rhesus monkey also will show prolonged blindness if the original lesion is expanded to include most of the LGB cortex. Three animals prepared in this way have thus far remained blind for a median period of 100 days with no signs of recovery. It is important to note that the lesion was alone alone cause this effect as evidenced by two monkeys with such a limbic ablation that demonstrated accurate visual guidance immediately after surgery.

We have proposed on the basis of other evidence that the blindness results from a failure of visual processing within the intact visual system due to the loss of an essential activation normally provided, directly or indirectly, by the territory included in the ablation (Neurosci. Abs. 4:639, 1978). The present findings strengthen this hypothesis by rendering highly unlike the alternative possibility that the blindness reflects some transient state such as surgical shock or dissection. We can now test our hypothesis directly by examining stimulus-evoked neuronal activity within the intact visual system of chronically blind animals. (Aided by NEI fellowship 9F32EY-03292.)

We conclude that PGO waves and the PGO burst neurons code eye movement information. The data is consistent with the hypothesis that the PGO burst neurons transmit eye movement information to the visual system due to the loss of an essential activation normally provided, directly or indirectly, by the territory included in the ablation (Neurosci. Abs. 4:639, 1978). The present findings strengthen this hypothesis by rendering highly unlike the alternative possibility that the blindness reflects some transient state such as surgical shock or dissection. We can now test our hypothesis directly by examining stimulus-evoked neuronal activity within the intact visual system of chronically blind animals. (Aided by NEI fellowship 9F32EY-03292.)
A QUANTITATIVE MORPHOMETRIC ANALYSIS OF TRANSNEURONAL CHANGES FOLLOWING LONG-TERM DENERVATION OF THE OPTIC TECTUM IN XENOPUS

Jeanette J. Norden and John A. Freeman. Department of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232

The effects of denervation on post-synaptic tectal cells of metamorphic Stage 66 Xenopus laevis were investigated using standard anatomical and stereological methods derived from stereology. Differences in the number of cells present and in the nuclear volume of cells in Layers 8 and 9 between denervated and control tects were used to indicate transneuronal effects following short-term unilateral enucleation (30 days), long-term unilateral enucleation (120 days), and following a single optic nerve crush (150 days). Changes in the number of cells present were determined by computing an areal density (number cell nuclei/µm²) or by computing the volume fraction (Vv) of nuclei in Layers 8 and 9. Nuclear volumes were determined from measurements of the mean diameter of cell nuclei (under oil immersion optics).

Following long-term (120 days) enucleation, there is a decrease of 18% in the number of cells present in the tectum contralateral to the eye removal compared to the control (ipsilateral) tectum. Furthermore, there is a drop of 45% in the nuclear volume of the cells which remain in the denervated tectum, in addition to a substantial loss in total tectal volume. These results are consistent with experiments using other techniques such as uptake of [3H]-thymidine after injection. By contrast, no significant changes, except in tectal areas border the 3rd ventricle. They migrate to positions medial and toward the lateral geniculate body where they remain during the first third of growth. Subsequent expansion of the tectum occurs after all of the above have been expanded and the retina towards the lateral geniculate where they remain during the first third of gestation. One study has suggested that the retina grows at the same rate as the tectum and that the retina is not influenced by the eye being present (Shatz and Rakic, 1976). However, the present results indicate that the retina is not a major source of input to the tectum and that it is not a major source of input to the tectum.

Recent studies have indicated that the retina is not a major source of input to the tectum and that it is not a major source of input to the tectum. These studies suggest that the retina is not a major source of input to the tectum and that it is not a major source of input to the tectum. Therefore, the retina is not a major source of input to the tectum and that it is not a major source of input to the tectum. Therefore, the retina is not a major source of input to the tectum and that it is not a major source of input to the tectum. Therefore, the retina is not a major source of input to the tectum and that it is not a major source of input to the tectum.

The superior colliculus in tree shrew (Tupaia glis) appears to play an important role in form vision. Following unilateral colliculotomy, the superior colliculus shows a significant reduction in the number of cells present and the nuclear volume of cells in Layers 8 and 9. This reduction is most pronounced in the ganglion cells and the external plexiform layer, and is least pronounced in the internal plexiform layer and the optic layer. The superior colliculus in tree shrew is characterized by a significant reduction in the number of cells present and the nuclear volume of cells in Layers 8 and 9. This reduction is most pronounced in the ganglion cells and the external plexiform layer, and is least pronounced in the internal plexiform layer and the optic layer.

The superior colliculus in tree shrew is characterized by a significant reduction in the number of cells present and the nuclear volume of cells in Layers 8 and 9. This reduction is most pronounced in the ganglion cells and the external plexiform layer, and is least pronounced in the internal plexiform layer and the optic layer. The superior colliculus in tree shrew is characterized by a significant reduction in the number of cells present and the nuclear volume of cells in Layers 8 and 9. This reduction is most pronounced in the ganglion cells and the external plexiform layer, and is least pronounced in the internal plexiform layer and the optic layer.
DISTRIBUTION, SIZE, AND MORPHOLOGY OF RABBIT RETINAL GANGLION CELLS. Clyde W. Oyster and Ellen S. Takahashi. School of Optom. University of Alabama in Birmingham, Birmingham, AL.

As determined from extensive cell counts in flat-mounted retinas, ganglion cell density can be shown to vary about ten-fold from peripheral retina to the region of highest density. Even so, the maximum density is only about 5000 cells/mm². The peak is more of a ridge, extending some 90° along the center of the visual streak, while the region within which the cell density is greater than 2000 cells/mm². Although there is some elevation in cell density in the central and temporal end of the streak, there do not seem to be any clearly defined local regions of especially high cell density. Measurement of the total retinal area and areas enclosed by isodensity contours allowed estimates of total cell population to be made; the mean estimate for three retinas was 406,000 ganglion cells, a figure which agrees well with recent counts of the number of axons in rabbit optic nerve. Like previous studies, we found that large ganglion cells form a large portion of the cell population in peripheral retina than in the streak, while the reverse was true for small ganglion cells. These differences were not large, however; for cells in a given size range, the proportions changed only 10% or so between streak and peripheral retina. The superior retina appears to be different; there are considerably more large cells here than in other retinal regions having comparably low cell density (< 500 cells/mm²). Golgi stains show the rabbit ganglion cells to have a number of characteristic dendritic ramification patterns. Some are predominantly unistratified, with dendrites confined to either the scleral or vitreal sublaminar of the IPL. Others ramify throughout the thickness of the IPL and, like the unistratified groups, show several variations in the complexity of the branching patterns. An obvious group of ganglion cells are bistratified, a configuration which is consistent with the notion that they are on-off cells. Some one-fifth to one-fourth of the stained cells have the bistratified morphology, a proportion which is similar to the frequency with which on-off direction-selective cells have been recorded in rabbit retina. 

(Supported by USPHS Grant EY02207).


The choroidal circulation accounts for 85% of the total ocular blood flow. In comparison with the circulation in the renal cortex the choroid has four times the volume of blood flow per 100 grams of tissue. The question arises as to whether the high choroidal circulation is functioning solely to supply nutrients to the outer avascular retinal layers. The exceptionally high oxygen content of venous choroidal blood, about 95% of that found in arterial blood, would seem to suggest that another role might be the dissipation of heat generated by the absorption of focused light in the outer retinal layers and the retinal pigment epithelium.

To study this hypothesis temperature measurements were taken from the macula of the cynomolgous monkey eye. A thermistor probe was focused light in the outer retinal layers and the retinal pigment epithelium.

Raising the intraocular pressure with the animals exposed to the 1.09 mW/cm² light source showed an increase in macular temperature when the intraocular pressure was elevated. Temperature measurements were also taken from a peripheral retinal site. Increasing the intraocular pressure here produced a decrease in temperature (irreceptive of the light source. The decrease in temperature was slightly less marked with the 1.09 mW/cm² light source, but ran a parallel course with the changes observed with only background illumination. We conclude that in low light environments the choroidal circulation acts as a heat source, maintaining a constant temperature environment for the retina and the retinal pigment epithelium. In high light environments the choroidal vasculature switches to a "heat sink," dissipating the heat generated by the absorption of focused light at the macula.

RECEPTIVE FIELD STRUCTURE IN STRIATE CORTEX OF CAT. Larry A Palmer and Thomas L Davis*. Department of Anatomy, University of Pittsburgh, Pittsburgh, PA.

We have used 3 methods to define the receptive field structure of striate cortical neurons in the cat: FST responses to moving narrow bars, moving edges, and moving slit. Best insight into field structure were obtained with a combination of response planes and edge analysis. The receptive fields of excitatory and inhibitory domains in response planes of simple and complex cells. Simple cells always have spatially non-overlapping regions of excitation and inhibition (spatially homogeneous response planes). Complex cells, on the other hand, often exhibit the same sequence of excitation and inhibition at all points in the receptive field (homogeneous planes). Based on their planes, 2 distinct types of complex and 5 distinct simple cell types have been discerned.

Field structure as defined in the response plane can be used with most cells to quantitatively predict the results of moving slit or edge analysis. This was accomplished by staggered addition of the FSTs computed from each of the 2 primary excitatory domains in their planes. Simple cells show a single light edge discharge center corresponding to one single excitatory domain by definition. The most common simple cell has spatially offset light and dark edge discharge centers which correspond to two similarly offset excitatory domains. One edge is light off and one at light on. In all these cells, the strengths and positions of inhibitory domains modify responses to moving edges and slits. While staggered addition of FSTs of a response plane usually quantitatively predict symmetrical or mildly asymmetrical responses to moving stimuli, it usually fails to predict direction selectivity.

We regularly find 8 distinct types of striate neurons based on receptive field structure alone. We anticipate that this physiological diversity will help to account for the variety of neuron morphologies recently described with serial electron microscopy (Davis and Sterling). NSF Grant BMS-78-25197.


The property of binocularity in visual cortex neurons has been assumed to depend upon connectivity of fibers originating in adjacent lateral geniculate laminae. This study indicates that the corpus callosum is essential for normal binocularity. Following section of the posterior corpus callosum of the cat, units were recorded from area 17 in cats two or more days after surgery. The response properties and ocular dominance of each cell were determined as were the single units of the primary receptive fields. The extent of the lesion was verified histologically after the final recording session. One sham operated and five normal cats were studied for comparison.

In normal and sham-operated cats over 80% of the neurons are binocularly driven. In posterior corpus callosum sectioned cats the proportion of such cells decreases to 55%. Surprisingly this effect is not limited to cells which have receptive fields in a narrow band adjacent to the vertical meridian but can be found up to 30° lateral.

Other properties such as the proportions of simple and complex cells sampled in area 17 were unaffected by the lesion. A reduction in binocularly driven cortical cells has been shown in cats given surgically induced strabismus or monocular suture prior to the time of natural eye opening. Such cats also have abnormal callosal connections (Lund; Innocenti and Frost). These data and our evidence on the role of the corpus callosum in binocular integration suggest that disruption of the normal development of callosal connections may underly the loss of binocularity following early visual deprivation and strabismus.

Supported by grants EYO02488-01, EY-2088-01 and RRS772923.
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The dorsal lateral area (DL) is one of the five visual field representations that underlie the anterior border of V-II and collectively constitute the third tier of visual areas in the owl monkey. DL wraps around the Middel Temporal area (MT) and relates to it topographically much as V-I does to V-II. We have qualitatively studied the response properties of 54 neurons in DL to stimuli of different size, direction of movement, and orientation. 21 of the 54 neurons showed a striking size selectivity; these cells responded only to a narrow range of preferred sizes, and the preferred sizes were often smaller than the receptive field. Other units exhibited spatial summation up to a certain stimulus size, but were decreasingly responsive to larger stimulus. Neurons with similar size preferences were encountered sequentially in penetrations made normal to the brain surface. We have also observed qualitatively that many of these neurons were very responsive to expanding and/or contracting stimuli around the preferred size. The response properties of size-selective cells were largely independent of stimulus intensity and contrast.

DL neurons differ from cells in other extrastriate areas in their directionality and tuning to moving bars and spots. Directionality was quantified by comparing the response in the best direction to response in the opposite direction. Tuning was measured by comparing the response in the best direction to the response in the surroundings in directions within ±90 degrees.

DL neurons were compared to neurons in MT and in a medial group of third tier areas, the Dorsomedial (DM), the Posterior Dorsal (PoD), and the Medial (M) areas. Cells in the medial group of areas are strongly orientation selective because they exhibit sharp tuning to bars and weak directionality. Medial group neurons are much more narrowly tuned to bars than to spots. MT neurons are strongly directional and only marginally more narrowly tuned to bars than to spots. DL neurons are more broadly tuned than MT neurons but much more directional than neurons in the medial group. DL neurons are only slightly more tuned to bars than to spots. In many cases, however, the most remarkable property of DL neurons is their selectivity for size stimulus.

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Behavioral tests of the compass orientation of houseflies (Musca domestica) under different lighting and magnetic field conditions have been carried out in order to test the theory of the mechanism of magnetic field reception proposed by Leask (Nature 267: 144, 1977). This theory implicates visual receptors capable of detecting the phosphoretic emission of a pteridine pigment(s) present in the visual cells. Results of the present study on the orientation of houseflies 1) the magnitude of the magnetic compass bias, 2) a dependence on the axis, but not the polarity, of the magnetic field and 3) an influence of the wavelength of incident light. Supported by EY07031, D.Y. Teller, Principal Investigator.


We have examined the projections from the lateral posterior (LP)-pulvinar (P) complex to several extrastriate visual cortical areas that were defined by electrophysiological mapping studies in this laboratory (Palmer, et al., J.Compr.Neural.177:237-256, 1978). Our method was first to label with horseradish peroxidase (HRP) and tritiated amino acids into that cortical site. An analysis of our HRP results show first that there are at least three representations of the visual field in the LP-P complex that roughly correspond to parcellation schemes based upon cortical and subcortical afferent zones (Updyke, J.Compr.Neural.113:322-323, 1972). Using the terminology of Updyke, we find that small injections into the area centrals (AC) representation of both areas 21a and PLS produced bands of labeled cells in the rostral portion of the LP-P complex. The label resulting from the area 21a injection was equally dense along the lateral border of P and the common border between LP and PLS; after the area PLS injection, the label was situated in the lateral half of PLS. Injections in the peripheral field representation of the horizontal meridional (HM) in area 20a led to labeling along the P-PL border and the medial half of PLS through-out the rostro-caudal extent of the LP-P complex. Second, concerning the representation of the upper and lower fields in the LP-P complex, an injection in the lower field representation of areas AMLS labeled neurons in the ventral portion of LP, whereas an injection in the representation of the upper field in areas VLS produced equal amounts of labeled cells across the dorsal portions of LP and PLS. Third, HRP results suggest that a great proportion of the LP-P complex is devoted to the AC and HM portion of the visual field. Further experiments will refine our understanding of cortical subdivisions. Some cortical areas, such as PLS, receive input from only one of the three thalamic zones (LP), whereas other areas such as anterior and posterior LP (P) receive input from all three. Preliminary comparisons of thalamic and corticothalamic connection patterns indicate the presence of reciprocal connections in every case. Finally, every area in the visual system thus far examined receives a bilateral input from the claustrum and an ipsilateral input from the central lateral nucleus. Supported by 1 R01 EY02654 and EY 132 0J 0735.
DORSAL LATERAL GENICULATE ORGANIZATION IN TURTLES, PRESDENT
Dorsal lateral geniculate complex is the dorsal thalamic region that receives primary retinal input in pond turtles. This complex has two structural components: a cell lamina (CL) and a neuropil region (N). N is a cell-poor region medial to the optic tract (OT). Frequency histograms of some areas from CL and N reveal significant differences in areas between regions, such that N neurons tend to fire more slowly. Autoradiographic studies of the optic nerve show that optic nerve axons are distributed throughout N. When these axons are anterogradely filled with HRP, it appears that 

Most dendritic segments in these fields are oriented perpendicular to OT fibers. Both types of distal segments may add, on many CL neurons. This type consists of one or more shaped arbors. Appendages with less complex arborizations are seen within N. Most of the collaterals are so thin that their diameters cannot be resolved. CL is medial to N. Cortical and, possibly, telencephalic inputs terminate in this component. CL can be cytoarchitecturally divided into rostral and caudal regions. Rostral CL neurons have densely packed somata. Many of these contribute to tightly packed clusters. Caudal CL neurons have less densely packed somata. Analyses of Golgi material, and of neurons solidly filled with retrogradely transported HRP, reveal two cell types, distinguished by both anatomical and functional criteria. CL neurons display at least one of two types of dendritic arborizations. The characteristic arbor type consists of sparsely branching dendritic trunks that form tufted or ball-shaped arbors. Appendages with less complex arborizations are scattered sparsely along more proximal segments of dendrites within N. A second type of collateral is found in addition, on many CL neurons. This type consists of one or more relatively thin dendritic segments that originate from the soma or are proximal dendritic trunks and extend within N. These neurons form the second type of geniculate neuron. Their dendritic arborizations are sparsely branched and oriented parallel to optic tract fibers. These arborizations usually do not project into CL. (Supported by PHS Grant GM-00094 and PHS Grant NS-12518.)

AN ASCENDING VESTIBULO-RETICULO-MLF PATHWAY IN CAT.
This electrophysiological study indicates that functional excitatory pathways exist from both vestibular nerves (V-nerves) through the vestibular nuclear complex (VN) and then to the pontine reticular formation (PRF) rostroventral to the abducens nucleus (MVN) was stimulated near its ventral border in the MLF, 77 could be orthodromically excited from the contralateral V-nerve (latencies: 1.8 to 7.3; av., 3.8 ± 1.4) and 31 neurons antidromically excited from both VN and the MLF, 77 could be orthodromically excited from the contralateral V-nerve (latencies: 1.65 to 6.9; av., 3.2 ± 1.2). In 5 of these experiments both V-nerves were also electrically stimulated at a rate of 50 Hz and no neuronal activity resulting from this stimulation was observed. The > 0.4 msec conduction time for vestibuloreticular neurons suggests that some vestibularly-related MLF fibers originate in the vestibular nucleus (synapse), then to the pontine reticular formation (PRF) rostroventral to the abducens nucleus (MVN) were stimulated. This vestibulo-reticulo-MLF pathway might participate in the visual system. Measurements of HRP into one optic tract demonstrate that all groups in nasal retina project to the contralateral hemisphere most of cells were recorded. Of a large field potential recorded around the perimetr of the optic disc in response to stimulation of the optic chiasm and the ipsilateral or contrala trans retinals reveals at least 3 conduction velocity groups among retinal ganglion cells axons retinal origines and central projections suggest that they are the axons of the 3 larger cell size groups seen in retinal white matter. Although the basic organization of the retinal ganglion cell population is similar in the cat and fox, the functional differentiation of this population into distinct groups seems more complete in the fox. (Supported by NIMH Predoctoral Fellowship F31 MH07204-01 to D.N.R. and NIMH Grant RO3 MH31459-01 to M.R.R.)

The overall pattern of retinal ganglion cell distribution in the gray fox, as seen in nissl-stained whole mounts, is quite similar to that in the cat. There are two retinal layers and a conspicuous horizontal meridian. However, the area centralis of the fox seems somewhat less developed than in the cat. Ganglion cells in the fox retina range in size from 15 to 20 µm, and the smallest axons are from 4 distinct size groups: 8-15, 16-23, 24-32 and 33-45 µm. Each of these 4 groups has a distinctive pattern of retinal distribution. In all regions of the gray fox retina, ganglion cells exhibit a wide range of ganglion cell sizes. A large fraction of ganglion cell axons have similar sizes and substantially the same distribution. The ganglion cells of the gray fox retina are more homogeneous than those in the cat retina.
It has been well established in a wide variety of mammalian species that the lateral retinal projection to area 19 is closely related to aberrant retinofugal projections. In general, it has been shown that the number of optic fibers decussating in the chiasm is abnormally increased in large portions of the albino rabbit decussates in the albino rabbit. Although much detailed work has been done on the retinogeniculate organization relatively few studies have focused upon the retinogeniculate pathway. The present study examines the differences in the ipsilateral optic projection to the superficial layers of the superior colliculus in albino and fully pigmented rabbits. Three albino and three wild type (fully pigmented) rabbits received intravitreal injections of 30-proline and allowed to survive for three days. Upon sacrifice, the brains were removed and processed for autoradiography. Although the fully pigmented rabbits received a heavier ipsilateral retinal projection than the albino rabbits, in both types of the animal the grains formed distinct clumps or patches and were restricted to the ventral part of the stratum griseum superficiale. In the fully pigmented rabbit the patches appeared in one to multiple to area 19. These patches appeared in one to multiple to the superficial layers of the superior colliculus in all areas of the STS. Connections from the ventral part of area 18 are directed to progressively more rostral portions of the STS. Connections from the dorsomedial part of area 18 terminate in the caudal most part of the sulcus, predominantly in the lower bank and depth of the sulcus.

Connections from peristriate cortex (area 18 and 19) to STS considerably less information is available in visual processing and in short-term memory. The post-trial activity of some neurons may represent reaction to interoceptive feedback on the monkey's performance. The remainder of the cells displayed differential activity related to the color or position of the cue. However, these revealed a variety of binocular interactions. For half of the cells, diffuse light stimulus to one eye was excitatory, whereas for the other eye it was inhibitory.

**DIFFERENCES IN THE IPSILATERAL RETINOCOLICULAR PROJECTION IN ALBINO VERSUS FULLY PIGMENTED RABBITS.**

Michael Rossa, Dept. of Anatomy, University of Illinois at the Medical Center, Chicago, Illinois 60680.

It has been well established in a wide variety of mammalian species that the lateral retinal projection to area 19 is closely related to aberrant retinofugal projections. In general, it has been shown that the number of optic fibers decussating in the chiasm is abnormally increased in large portions of the albino rabbit decussates in the albino rabbit. Although much detailed work has been done on the retinogeniculate organization relatively few studies have focused upon the retinogeniculate pathway. The present study examines the differences in the ipsilateral optic projection to the superficial layers of the superior colliculus in albino and fully pigmented rabbits. Three albino and three wild type (fully pigmented) rabbits received intravitreal injections of 30-proline and allowed to survive for three days. Upon sacrifice, the brains were removed and processed for autoradiography. Although the fully pigmented rabbits received a heavier ipsilateral retinal projection than the albino rabbits, in both types of the animal the grains formed distinct clumps or patches and were restricted to the ventral part of the stratum griseum superficiale. In the fully pigmented rabbit the patches appeared either in excitation or in aggregates of two to three in the albino rabbit. On the other hand, only the diffuse light stimulus to one eye was excitatory, whereas for the other eye it was inhibitory.
2720 LECTIN-BINDING TO ISOLATED CELLS FROM TURTLE RETINA. P. Vijay Sarthy*, C. David Bridges*, Francis L. Kretzer* and Dominic H.C. Lam. Callen Eye Institute, Baylor Collene of Medicine, Houston, TX 77030.

The presence of specific saccharides on the surface of retinal cells was examined by reaction FITC-labeled lectins with cells dissociated from papain-treated retina. The pattern and intensity of binding was found to vary among the cells examined. With Con A (α-mannopyranosides, α-glucopyranosides and oligomers of mannose) there was strong surface binding to both rod and cones, with an intense ring of fluorescence just above the nucleus. Bipolars also showed strong surface labeling with intense labeling on the soma and at the synaptic ending. The pattern seen with Müller cells was quite striking in that intense fluorescence was observed in the axonal, microvillus region. In contrast, the horizontal cells were devoid of any surface label. These observations are in agreement with the binding of ferritin-Con A seen in sections of turtle retina. When 9icin-60 (β-d-mannopyranoside) or 9icin-62 (β-d-maltopyranoside) or whey eam annulin (oligomers of α1 → 4 linked N-acetyl glucosamine) were incubated with photoreceptors (devoid of outer segments), binurals or horizontal cells, little or no fluorescence was visible. However, all the three lectins bound strongly to the apical portion of the Müller cell. Asparagus pea lectin (α-L-fucopyranosides) did not bind to any of the cells examined, although patches of amorphous fluorescent material were often seen. In all cases examined, lectin-binding was inhibited by the appropriate haptene sugar: Me-α-Man for Con A, GalNAc for RCA 60, Gal for RCA 120 and Sarthy*, C. David Bridges*, Francis L. Kretzer* and Dominic H.C. Lam. Callen Eye Institute, Baylor Collene of Medicine, Houston, TX 77030.


Intracellular recordings have been made for the first time from the photoreceptors of the compound eye of the branchiopod crustacean Daphnia magna. Cells of all types, 176 of which form 22 fused rhadomys, are about 10µm in diameter and 30µm in length. Their axons project some 100µm to the optic ganglion, where they synapse onto 110 lamina neurons. These synaptic projections have been mapped in detail (PNAS 70: 57, 1973).

The photoreceptors have resting potentials in the range of −30 to −50 mV and slowly depolarize when illuminated. These depolarizations are composed of a transient phase and a maintained phase and are generally similar to other invertebrate photoreceptor potentials. Both the size and shape of the potential and its latency are strongly affected by the state of adaptation of the receptor and by the intensity of illumination. The transient phase is especially reduced in duration in the light-adapted state, revealing a spike-like response. There is, however, no evidence of impulse activity nor can the spike-like response be evoked by current injection in the cell body. Although Daphnia have been reported to show visual behavior, the implied visual function and synaptic transformations have not been established. Although neuronal recordings have been reported from the lamina of the eye of Daphnia, no visual responses were observed, and no evidence of impulse activity was noted in these recordings. The results of this study extend the range of visual phenomena to the adult Daphnia, and suggest that the photoreceptor lamina of Daphnia is capable of encoding visual information. This is in agreement with the results of the visual behavioral experiments on Daphnia (Schehr, 1970).
DISTRIBUTION OF NEURONS CONTAINING CYTOPLASMIC LAMINATED BODIES (CLB) IN THE dLGN OF MONOCULARLY DEPRIVED (MD) AND DARE RARED (DR) CATS. Harri Luis Schmidt, Union College, Schenectady, N.Y. 12308.

Large samples of cells of the cat's dLGN including the projection area centrals in lam.A, A and the monocular segment (MD) were examined for the presence of CLB in the present sections. Cells were stained with Luxol fast blue and nerve cells with Carboll fuchsin or Cresyl violet.

Considerable variation in the proportion of CLB containing cells among nearby regions of the same dLGN as well as among corresponding regions from different normal cats had been described (Schmidt, MacNichol, Absch. 1977). The mean percentage of CLB-containing neurons in four cats vary between 40-61% in lam.A(medial), 49-33% in lam.A and 46-47% in the MS.

In lam.A(area), the distribution of CLB-containing neurons was observed in four dLGN of MD cats: lam.A(medial) 46-66%, lam.A 42-53%; MS 33-55%. The proportion of CLB-containing neurons in lam.A of the individual dLGN was either equal or lower than in lam.A(medial) regardless of which lamina had input from the deprived eye. A high or low proportion of neurons containing CLB's in the MS corresponded to a high or low percentage of cells in lam.A. Furthermore, a few portions of laminae and AI in the particular dLGN. The percentage of nerve cells containing CLB's in two dark reared animals are in the range found in normal animals: lam.A(medial) 62 and 58%; lam.A 55 and 50%; MS 42 and 48%.

A very small percentage of neurons in the dLGN of normal cats contain either two or three CLB's. A high proportion (70%) of the posterior per cent of these cells. There may be a true increase in the number of CLB-containing cells in normally deprived animals. Moreover, it appears that cells containing CLB's serve more frequently in these cats since their nerve cells may be smaller due to deprivation.

A recent study indicates a significant change in the proportion of CLB-containing nerve cells in the dLGN after visual deprivation as would be expected when CLB serves as markers for X-cells (Cathy & Frerker, J Comp Neurol. 1712577), 563-594). However, very few or no CLB cells have been recorded in either the normal or visual deprived cats. Three interpretations are possible: (1) CLB are not found in either X- or Y-cells exclusively or (2) only a very small fraction of Y-cells are lost or (3) Y-cells are not lost due to visual deprivation but the probability of recording from these cells is decreased.

Support provided by NIH Grants R01 EY-01268 and R01 EY-02892 and A.P. Sloan Foundation Fellowship 1677.

INTERHEMISPHERIC CONNECTIONS OF RETINOPHICALLY DEFINED VISUAL CORTICAL AREAS IN THE CAT. Mark A. Segmures, Dep. Anat., Univ. of Pa., Phila., PA 19104.

The distribution of visual cortical cells whose axons pass through the corpus callosum was examined using HRP and/or anterograde tracing. The posterior part of the callosal was cut and a cotton pledget soaked in 5% HRP placed between the cut ends of the callosum for survival periods of 48 hours. HRP labelled axons were found along the entire 17/18 border and the lateral half of area 19. A comparison with the histotopic maps of Palmer and Kelly, 1970, indicated that labelled cells in 17 were confined to an estimated 5° from the vertical meridian (VM). Cells in 19 were labelled to about 10° from VM in 19. In contrast, extensive label was present in nearly all portions of lateral suprasylvian (22,17,18,19) and 21. Furthermore, callosal cells in these areas appeared to convey information from a more widespread portion of the visual field, out to as much as 40° from VM. Callosal cells at the 17/18 border and in 19 were primarily restricted to lower layer III (50%) and upper layer IV. Medium to large pyramids were found in both laminae and a few stellate cells were labelled in IV of 17 and 18. A small percentage of label in 19 consisted of pyramidal and fusiform cells in layers V and VI. In LG areas, medium to large pyramids in III still accounted for about 60% of labelled cells, but there was a decrease in the number of cells in IV and a substantial increase in the number of labelled cells in V and VI.

A number of small, combined HRP-tritiated leucine injections were made from a recording microejet at electrophysiologically defined cortical injection sites. Injection sites included a range of receptive fields from local to broadly tuned to VM. It was found that injections restricted to specific areas were 20, and 21 always received the highest callosal input from the homotopic cortex of the contralateral side; thereby connecting visual fields with the homotopic cortex of the contralateral side. All visual cells in the area 17 were mirror symmetrical in the case of injection sites located away from VM. In addition, there was no convergence of projections from the injection site from other visual areas know to contain callosal cells in visual field representations that were mirror symmetrical to the receptive field of the injection site. (Supported by 1801 EY02564 & 5701 GM00281)


In recent work, it had been shown that several visual illusions (Nackay after-image, fortification illusion) may be explained in terms of the existence of spatially tuned columnar visual mechanisms in the primate visual field (Schwartz, 1979). In previous work, it had been shown that the global retinotopic map, and the size of cortical ocular dominance columns varied as a function of binocular disparity (Schwartz, 1977) which was subsequently verified (Schwartz, 1979). The implication of this work is that the visual cortex is capable of expressing a spatially tuned functional organization. The present paper is extended to show that regular interlaminar and reciprocal connections are sufficient to encode the difference map of the retinotopic receptors as a spatial frequency modulated signal. Binocular disparity and color opponency are examples of visual sub-modalities which are represented by columnar structure at the cortical level, which by definition are difference maps of discrete receptor systems, and which are known from psychophysical work to have differential spatial frequency sensitivity. It is shown by analytic calculation, and by digital and optical simulation, that columnar spatial filtering are sufficient to provide segmentation (feature extraction) of the visual scene based on spatial texture of the cortical map, for color and depth channels. Visual texture may be extracted by filters capable of encoding differential color, depth, and spatial frequency filtering. The existence of well defined neuronal trigger features is consistent with this analysis, and in fact, may be quantitatively derived. However, segmentation (feature extraction) may be conceptualized entirely in terms of spatial-temporal pattern, with no reference to the discrete cellular properties of the nervous system, beyond the receptor level. This provides a field theory of vision which is atomically, physiologically, and psychophysically consistent.

Schwartz, E. L. Vision Research (1979)

VISUAL-VESTIBULAR INTERACTION IN HEMIDECORTICATE HUMANS.

James A. Sharp and Alex V. Lo". Div. Neurology, University of Toronto, Toronto, Ont. (Med. Ctr.

Visual modulation of the vestibulo-ocular reflex (VOR) was quantitated in 5 subjects 8 to 12 years after cerebral hemidecortication for intractable epilepsy of the temporal lobe. Data were compared with data from 14 control subjects. Horizontal vestibular smooth eye movement velocity/velocity gains were measured during passive horizontal voluntary whole body rotation at frequencies from 0.3 to 1.0 hertz. While attempting fixation of a target moving with the head, hemidecorticate patients showed normal human horizontal vestibular smooth eye movement contralateral to the side of cortical removal at each frequency. During attempted fixation of a stationary target, the VOR gain was found to be abnormally low at normal rotational frequencies so that eye velocity failed to match head velocity. The impaired visual suppression of the contralateral VOR while fixating targets moving with the head and the defective augmentation of the ipsilateral VOR while fixating stationary targets were compensated for by saccadic movements toward the side of cortical ablation. In darkness, VOR gains were horizontally symmetrical and normal.

With the subjects' heads immobilized to eliminate vestibular eye movements, smooth pursuit of 20° amplitude target motion was examined at 8 frequencies, from 0.125 to 2.0 hertz. During tracking toward the decorticate hemisphere smooth pursuit velocity/target velocity gains were subnormal at target frequencies over 0.125 hertz. During tracking away from the side of cerebral cortical ablation, gains exceeded unity at target frequencies up to 0.75 hertz. With the head immobilized each subject had primary position jerk nystagmus toward the decorticate hemisphere. The nystagmus stopped in darkness.

The horizontal VOR in darkness and failure of the nystagmus slow phase velocity to increase in darkness implicated the horizontal pursuit system imbalance in the genesis of the fixation nystagmus. The horizontal pursuit of light correlated with the impaired visual augmentation of the ipsilateral VOR and with the impaired visual suppression of the contralateral VOR. These enduring manifestations of hemidecortication demonstrate the important role of the cerebral hemisphere in visual-vestibular interaction.

Supported by MRC of Canada grants ME 5509 and MA 5404
EFFECTS OF STRIATE CORTEX AND/OR SUPERIOR COLLICULI ABLATIONS ON number of correct responses to contralateral target locations.

With previous findings do not support a sharp dichotomy between and, since the reaction product is contained within cells, allows different lectins in one animal. Compared with the autoradiography of 125I-lectin the immuno-peroxidase method is faster thickness. (Supported by 1 F32 EY 05296-01 and EYRO-1960.)

The lesions were histologically verified. The task, given under normal illumination, required the animals to reach for a target randomly placed in one of 8 equal segments of a 4.6 cm diameter while disc located centrally at 20 cm above the center of the table. An apple cube, affixed to the center of the target served as the reward for accurate reaching. A correct response consisted of an initial contact with either the target or the apple cube directly.

The animal was trained sequentially to a criterion level of performance using black discs of decreasing diameter (90, 55, 35 and 15 mm). Finally only the apple cube of about 10 mm was presented as the target. Six of the monkeys with only bilateral striatectomies could be retrained to reach accurately for all targets but with marked deficits in the upper quadrants. No deficit was noted on subsequent targets. An additional decrease in performance of similar magnitude occurred when the apple cube alone was presented. This time errors were greater in the lower quadrants. Four animals with bilateral colliculectomies performed just as accurately as before the operation. The other four subjects failed in two or more stages. In one of them, there was a transient increase in errors to targets on the right side after initial removal of the left colliculus. In two monkeys, however, also some accidental damage to the left occipital lobe.

The afferent connections of the SC to the remaining visual cortex have been described in a number of studies. The present findings indicate that the superior colliculi are disorganized and that the effective injection site was indeed restricted to the dense region of injection. Labelled cells were charted with an X-Y plotter, and in some cases the injection site was indeed restricted to the dense region of injection site should facilitate the study of local connections. Second, it offers the possibility of demonstrating multiple projections from a single region by injection of several different lectins in one animal. Compared with the autoradiography of 125I-lectins, the immuno-peroxidase method is faster and since the reaction product is contained within cells, allows an unequivocal identification of the injection site.

The method has two potential advantages over conventional HRP tracing techniques. First, the restricted nature of the injection site should facilitate the study of local connections. Second, it offers the possibility of demonstrating multiple projections from a single region by injection of several different lectins in one animal. Compared with the autoradiography of 125I-lectins, the immuno-peroxidase method is faster and since the reaction product is contained within cells, allows an unequivocal identification of the injection site.
THE SPATIOTEMPORAL ORGANIZATION OF X-, Y-, and W- CELLS.

Steven and Serstein have described two types of receptive fields (RFs) in cat retina: one group has a spatially heterogeneous distribution of excitation and inhibition, and the second group has a spatially heterogeneous distribution. In contrast, many researchers have investigated three basic groups: X-, Y-, and W-RFs. We have repeated the conventional tests for X-, Y-, and W-RFs and compared them directly to the heterogeneous/homogeneous characteristics of the cell described here.

All units classified as X-cells by conventional methods had heterogeneous-RFs. Mean latency from the optic chiasm (OC) was 3.20 msec. (s.d.0.05, n=5). At a reference stimulus, a bright flash on a small bright bar, of 0.5 to 6 deg/sec with the eye at 16 deg/sec. The spatial frequency at which these heterogeneous cells stopped their modulated response to drifting gratings was 1.94 c/deg or higher. Mean center size was 0.94 deg. (s.d.0.035, n=4). Cells with strong surround showed a clear null position to gratings.

All units classified as Y-cells had homogeneous-RFs. Mean latency from the OC was 1.16 msec. (s.d.0.23, n=5). Twenty-two of these cells could be activated from the superior colliculus (SC) and their OC latency was 1.12 msec. (s.d.0.18).

When a bar 1 by 0.25 deg. was used as a stimulus, these cells had velocity preferences ranging from 32 to 256 deg/sec, with the mode at 128 deg/sec. The spatial frequency at which these heterogenous cells stopped their modulated response to drifting gratings was 1.94 c/deg or higher. Mean center size was 0.94 deg. (s.d.0.035, n=4). Cells with strong surround showed a clear null position to gratings.

All data were collected from a new method developed by Steve and Serstein.

We have repeated the conventional tests for X-, Y-, and W-RFs and compared them directly to the heterogeneous/homogeneous characteristics of the cell described here.

Some preparations exhibit "spontaneous" quantum bumps whose frequency increase (up to ~ 20/min) during dark adaptation and are suppressed after a light pulse. Light sensitivity in dark adapted eyes was 1000 MPs from a single eye. These cells as well as others attached to the back of the eye and are sensitive to light with graded depolarizations over 12 hr. PRs respond to light with graded depolarizations using a programmer to scan the dynamic range of a PR, that light with 1000 MPs from a single eye. PRs can be recognized by a microvillous bush (rhabdomere) which marks the portion of the cell oriented toward the lens.

2734 DISSECTION OF A NEURONAL CIRCADIAN OSCILLATOR SYSTEM IN THE EYE OF APLYSIA: INTRACELLULAR RECORDING FROM SINGLE DISCONNECTED PHOTORECEPTORS IN CELL CULTURE. Felix Strumwasser, D. P. Viele*

2735 VISUAL CORTICAL INPUT TO AREA 20 OF THE CAT: ANATOMICAL EVIDENCE FOR SUBDIVISION OF THIS REGION INTO TWO AREAS. Laura L. Symonds and Alan C. Rosengquist, Dept. of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104.

Area 20 of the cat visual cortex was first defined by Hecht and Jones on the basis of the patterns of connections with cortical and thalamic regions. Recently, our laboratory has electrophysiologically mapped this region of the visual cortex and subdivided it into two areas called 20a and 20b. We have shown that areas 20a and 20b are somewhat unique among other cortical areas because they receive projections from three separate subdivisions of the lateral posterior complex. We have studied the cortical connections of areas 20a and 20b to determine the degree of retinotopy, the pattern of cortical connectivity, and whether anatomical evidence could be found to support the division of this region into two functional areas. Our method was to make small combined retrograde/horseradish peroxidase (HRP)/[3H]-leucine injections from a recording microelectrode at electrophysiologically defined regions of either 20a or 20b. The results reported here are based on the HRP data providing information about the cortical afferents to 20a and 20b. First, our results show that after injections into physiologically defined regions of 20a or 20b, the labelled cells in other cortical areas are located only at similar retinotopic loci. Second, both areas 20a and 20b receive projections from many of the same cortical areas, including areas 17, 18, 19, 21b, and 3. Third, both 20a and 20b receive projections from different lateral suprasylvian visual areas. 20a receives input from PLLS and DLS on the lateral bank; 20b receives input from the medial bank.

Our data thus provide additional evidence for the existence of two separate cortical visual areas within area 20 of the cat brain, confirming recent electrophysiological studies. In addition, although areas 20a and 20b are regions which receive converging input from the posterior thalamus, they also receive input from cortical areas....

2736 SUPERIOR COLLICULUS INFLUENCES ON EYE MOVEMENT IN THE NEONATAL CAT. Barry E. Stein, H. Peter Claussen and Stephen J. Goldberg, Departments of Physiology and Anatomy, Medical College of Virginia, Richmond, Virginia 23298.

The present experiments were initiated to (a) determine whether eye movements can be generated via the superior colliculus even before colliculus neurons are activated by visual stimuli, and (b) determine whether eye movements can be generated even before colliculus neurons are activated by visual stimuli. We therefore studied eye movements evoked in adult cats. Twenty-six kittens, 2-77 days of age, and six adult cats were studied. A steel chamber was fitted over a cranial opening in each animal and a small mirror was glued to each animal with vaseline (the eyelids were surgically opened when necessary). Electrical stimulation of the superior colliculus consisted of 70 msec. trains of 1000 pulses at 200 Hz, delivered through glass insulated tungsten microelectrodes. The electrodes were lowered to the colliculus through the visual cortex and eye movements were measured using the displacement of light beams which were reflected off each eye onto a translucent hemisphere positioned in front of the animal. Adult cats exhibited many spontaneous conjugate saccadic movements, as well as some conjugate drifts of the eyes. Evoked eye movements, however, were always contraversive and conjugate. Neonates (2-12 days of age), on the other hand, showed many spontaneous slow drifts of the eyes and few ballistic-like movements. Interestingly, the eyes often moved independently of one another spontaneously. With electrical stimulation, eye movements, as well as a variety of other movements, could be evoked in the young animals studied. However, the eye movements elicited in kittens were of smaller amplitude, sometimes conjugate and sometimes saccadic. The results reported here are based on the data collected from 27 kittens, in which the eyes were moved in different directions and at different times. The results reported here are based on the data collected from 27 kittens, in which the eyes were moved in different directions and at different times.
**2736**

**SIZE AND DISTRIBUTION OF RETINAL GANGLION CELLS PROJECTING TO THE RABBIT MEDIAL TERMINAL NUCLEUS.** Ellen S. Takahashi, Clyde M. Oyster, John J. Simpson and Robert E. Spodak. School of Optometry/University of Alabama in Birmingham, Birmingham, AL 35294 and Department of Physiology & Biophysics, New York University Medical Center, 55 First Avenue, New York, NY 10016.

Recent evidence from extracellular recording studies indicates that the medial terminal nucleus (MTN) of the rabbit diencephalon receives major, if not exclusive, inputs from a specific class of retinal ganglion cells, the on-type direction-selective cells. These ganglion cells have been selectively labeled by the retrograde transport of horseradish peroxidase (HRP) injected into the MTN. The number of labeled cells, their distribution over the retina, and their soma areas were determined. In one animal, in which the HRP injection completely filled the nucleus, some two thousand ganglion cells were labeled in the contralateral eye. This number agrees with previous estimates of the number of retinal axons terminating in the MTN. Unlike results in other species, none of the ganglion cells were displaced (Dopps's cells). The density of labeled cells was highest in the visual streak and, overall, the distribution of labeled cells corresponded with the regional distribution of on-type direction-selective cells as previously determined in electrophysiological studies. Cells labeled by the HRP injection were among the 20% largest cells in the retina. This result, in conjunction with conclusions from other studies, leads to the prediction that on-type direction-selective cells can be characterized morphologically as having large cell bodies, very extensive dendritic spreads, and dendrites which ramify in the vitreal sublamina of the inner plexiform layer.

Although the ipsilateral retinal projection to the MTN in pigmented rabbit, as demonstrated by autoradiography, is considerably less dense than the contralateral projection, the number of labeled cells (seven) in the ipsilateral retina was lower than expected. This difference may reflect the relative sensitivities of the retrograde and autoradiographic techniques. (Supported by NIH Grants EY 00771 and EY 02207).

**2737**

**MONOAMINE OXIDASE ACTIVITY IN RETINA-DISTRIBUTION AND DRUG INHIBITION.** Thomas N. Thomas, David L. Sparks*, Neil S. Buckholtz, and John W. Zeman. Department of Psychiatry, Medical University of S.C., Charleston, S.C. 29403

Dopamine (DA) and serotonin (5-HT) are putative neurotransmitters in the retina. Monoamine oxidase (MAO) catalyzes the enzymatic degradation deamination of these monoamines. Evidence points to the existence of at least two different forms of MAO (type A and B) having different substrate specificities and inhibitory sensitivities in the brain and other tissues. In brain, norepinephrine and 5-HT seem to be MAO-A specific whereas DA can be degraded by both MAO-A and B. There is very little information available regarding the types of MAO and drug sensitivities of MAO in retina. This report deals with the distribution of MAO A and B in bovine retina and their inhibition by a number of drugs known to affect brain MAO activity. Fresh bovine retinas were homogenized in ice-cold 0.32 M sucrose and fractionated to give two symptomatical fractions. One fraction (P1) was enriched in photoreceptor terminals and the second fraction (P2) contained symtoms derived from the inner plexiform layer. Type A MAO activity in these fractions was assayed using 1.5 nM [3H]-serotonin as substrate and type B with 4 nM [3H]-phenylethylamine.

The distribution of MAO activity (nmoles/hr/µg protein) in retinal fractions is shown in the table below (mean ± SD).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>0.67 ± 0.23</td>
<td>7.12 ± 0.21</td>
</tr>
<tr>
<td>P1</td>
<td>0.79 ± 0.26</td>
<td>9.53 ± 1.11</td>
</tr>
<tr>
<td>P2</td>
<td>2.92 ± 0.26</td>
<td>27.41 ± 0.84</td>
</tr>
</tbody>
</table>

The ratio of MAO-A to B activity is approximately equal in all fractions. Monoamines in retina have been shown to be localized in the inner nuclear layer and this evidence derived from this region contains most of the MAO-A activity. Therefore, the P2 fraction was used to determine the IC50 values of a number of monoamine oxidase inhibitors and solubilized α-malealamine and equal potency in inhibiting both MAO A and B. 6-Methoxy-1,2,3,4-tetrahydro-8-carboline and clorgyline were more potent inhibitors of type A, whereas pargyline and α-malealamine was more effective at inhibiting type B.

Similar actions of these drugs have been demonstrated on brain MAO except that, in brain, pargyline is a more potent MAO-B inhibitor. Drugs that inhibit MAO have proven useful in the treatment of depression and mild to moderate hypetension. Our observations raise the possibility that these drugs, by inhibiting retinal MAO activity, may alter retinal neurotransmission and possibly vision. Supported in part by P.H.S. Grant MO26712 (N.S.B.) and South Carolina General Medical and Faculty Research Appropriation 1978-79 (T.N.T., N.S.B.).


2 Thomas and Redburn Exp. Eye Res. 28 (1979) 55-61.

**2738**


In monocularly deprived (MD) cats, few cells in the visual cortex respond to input from the deprived eye, while most cells in the lateral geniculate nucleus (LGN) respond relatively normally. This suggests that normal visual cortex may be damaged by primary visual deprivation. The effect of secondary visual deprivation (SDV) on primary visual cortex (visual streak) was investigated. I found a shrinkage of ocular dominance patches from the deprived MD cortex.

**2739**

**LOSS OF GENICULOCORTICAL TERMINALS FROM DEPRIVED LAMINAE IN MONOCULARLY DEPRIVED CATS.** Suzannah Bliss Tieman, Neurobiology Research Center, State University of New York at Albany, Albany NY 12222.

In monocularly deprived (MD) cats, few cells in the visual cortex respond to input from the deprived eye, while most cells in the lateral geniculate nucleus (LGN) respond relatively normally. This suggests that normal visual cortex may be damaged by primary visual deprivation. The effect of secondary visual deprivation (SDV) on primary visual cortex (visual streak) was investigated. I found a shrinkage of ocular dominance patches from the deprived MD cortex.
EFFECTS OF BINOCULAR DEPRIVATION ON RESPONSES OF CELLS IN THE CAT’S LATERAL SUPRASYLVIAN VISUAL CORTEX. Lillian Tong*, U. Wisconsin, Madison, WI 53706.

It has been observed that, following surgical rotation of an eye, cats show evoked potentials and visual function measured in the rotated eye (cf Mitchell et al. Exp. Brain Res. 1976, 25: 109-113; Peck et al. Exp. Brain Res. 1979, 34, 401-418). Visuo-motor behaviour is also abnormal, showing patchy pattern discriminations and they appear to be able to compensate for their rotated visual world. The present experiments measured the visual acuity of cats which had undergone surgical eye rotation. Rotations were accomplished by sectioning the extraocular muscles and repositioning the globe within the orbit. Acuity was assessed using the jumping stand technique (Mitchell et al. 1976, J. Physiol. 181-193), in which the cats were trained to discriminate a high contrast grating pattern from a gray field matched for luminance.

One group of kittens underwent unilateral rotations of about 90° at approximately the time of natural eye opening. In all cases, when each eye was tested separately, the acuity of the rotated eye was significantly worse than that of the normal eye by a factor of 2 or more, even though visuomotor coordination through the rotated eye seemed quite adequate. Kittens which were given smaller degrees of rotation showed only correspondingly less severe deficits. A second group of kittens received unilateral rotations at 2 months of age or older. Those cats with very large rotations (around 90°) also showed severe deficits in the rotated eye and those with smaller rotations were less impaired. A final group underwent bilateral rotation of approximately 10°. Although there was some indication that acuity was reduced in both eyes of these cats, there was little difference between the two eyes.

Control experiments, anatomical and physiological observations, suggest that the deficits are not due primarily to physical disturbance of the visual pathways. It seems more likely that abnormal vision is a form of amblyopia produced by discordant binocular stimulation.

Supported by grants from the Medical Research Council of Canada (MA7125 to R.T.) and the U.S. Public Health Service (NS 14116 to C.K.P.)

EFFECTS OF BINOCULAR DEPRIVATION ON RESPONSES OF CELLS IN THE CAT’S LATERAL SUPRASYLVIAN VISUAL CORTEX. Lillian Tong*, Department of Psychology, University of Wisconsin, Madison, WI 53705.

Eight kittens were raised with unilateral lids sutured (BD), and single cells were recorded in the binocular and monococular segments of the lateral suprasylvian visual area (LS area). Only about 25% of the cells could be driven by visual stimuli in BD cats, compared to 87% of the cells in cats raised with binocular vision. Cells with SV drifting receptive fields generally responded better to stationary flashing stimuli than to movement (112), and none of the cells were direction selective. About 3% of the cells had inhibitory receptive field surrounds. In contrast, 95% of the responsive cells in normal cats have well-defined receptive fields and over 80% are direction selective. In addition, nearly 40% of the cells in normal cats have inhibitory receptive field surrounds. Thus, binocular deprivation produces severe abnormalities in the response properties of LS area neurons. In addition, cells in the binocular segment showed somewhat abnormal ocular dominance. Only 48% of the cells could be driven by the ipsilateral eye in BD cats, compared to 70% of the cells in normal controls. There was no difference in the effects of BD on receptive field properties of cells in the monococular and binocular segments of LS cortex.

Many of the abnormalities observed in the LS area of BD cats resemble those that are present in normally reared cats after visual cortex (areas 17, 18, and 19) have been removed (e.g., loss of direction selectivity, increased response to stationary flashing stimuli, and reduced input from the ipsilateral eye), while others differ (e.g., loss of responsive cells, increase in cells lacking well-defined receptive fields, and loss of inhibitory surrounds). To investigate the extent to which the changes following BD are due to abnormal cortico-cortical inputs, five additional BD cats were studied after visual cortex had been removed. There was no difference between the results in these animals (69 responsive cells were studied) and those in BD cats with visual cortex intact. These results suggest that the abnormalities observed in BD cats with visual cortex removed and those in normally reared cats with visual cortex removed indicate that binocular deprivation produces abnormalities in the thalamo-LS cortex pathway independent of the geniculo-striate system.

The cat’s dorsal lateral geniculate nucleus (LGN) is known to receive afferents from the superior colliculus (SC) as well as from the retina. It was of interest to study the precise relationship between these two pathways in normal cats. The role of the superior colliculus is known to differ according to different anatomical structures of the visual system. The present experiments demonstrate that different monocular areas of the visual system have different metabolic requirements in processing visual information. The role of the superior colliculus in visual information may be responsible for the greater increase in glucose utilization observed in this structure.
EVIDENCE FOR ADDITIONAL EXTRASTRIATE CORTICAL PROJECTIONS TO THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT. B. V. Udinie.

Department of Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana 70112

Substantial cortical projections onto the dorsal lateral geniculate nucleus have been shown to arise from visual areas 17, 18, and 19 in the cat. Areas 17 and 18 project onto all of the laminae and the intralaminar zones of the nucleus, while area 19 projects to the parvocellular C lamina (Udinie, 1973a: 1973b). These connections appear to reciprocate the known projections originating from the geniculate laminae (Gilbert and Kelly, J. Comp. Neurol., 163: 81, 1975).

In the course of investigating the projections arising from lateral suprasylvian areas, it was noted that certain of these areas also project onto the dorsal lateral geniculate nucleus. In these experiments, individual cortical areas - as defined by Tusa et al. (Soc. for Neurosci. Abstrs., 1: 52, 1975) - were identified by electrophysiological mapping, and then injected with tritiated proline. Projections onto the thalamus were identified autoradiographically.

No projections to the dorsal lateral geniculate nucleus were evident from areas AL5, PLS, and DLS occupying the lateral bank of the suprasylvian sulcus, or from area AMS on the medial bank of the sulcus.

Areas PM5 on the medial wall of the suprasylvian sulcus, and area 21a on the crown of the posterior suprasylvian gyrus project densely to the dorsal lateral geniculate nucleus. In addition, sparse terminal labeling of these laminae occurs after injections made more posteriorly on the suprasylvian gyrus. Because of the complex juxtaposition of areas 20a, 20b, VLS, and 21b in this region, it has not yet been possible to unequivocally identify the exact source of these corticogeniculate fibers. The responses of the unit monitored were recorded extracellularly as a function of the spot location. Spot sizes could be selected conveniently subtending angles from about 1° to 15° at the frog's retina. In this way the available field represented by the CRT screen could be scanned at different resolutions, with the finest scan consisting of 1024 field elements. The response matrices are translated into intensity patterns, and the global responses to these patterns are recorded. In some cases the patterns were modified by use of clustering techniques while observing the resulting responses.

Data will be presented of over 900 independent runs of about 70 cells. Most of the fields found were approximately circular with angular widths between 1° and 20°. Others were either elongated or showed irregular shapes. The majority of the cells showed a preference for dark stimuli on bright background rather than the opposite. When different resolutions were used, the results showed consistently that the responses of the different parts of the receptive field did not add linearly. Non-linear behavior and interactions between different areas of the receptive field were noted. The temporal characteristics of the responses were also examined; successive runs showed a high stability and reproducibility can be achieved over long periods of time.

(Supported by NIH grant EY02129)}

AREAL BOUNDARIES AND TOPOGRAPHIC ORGANIZATION OF VISUAL AREAS V2 AND V3 IN THE MACAQUE MONKEY. D. C. Van Essen, J. H. R. Maunsell* and J. L. Bixby.* Division of Biology, California Institute of Technology, Pasadena, CA 91125

The organization of areas V2 and V3 in the macaque was studied by analyzing cortical myeloarchitecture, projections from striate cortex (V1), and interhemispheric connections. Results were displayed on two-dimensional maps of parietal fields, calculated by positional measurements of spatial relationships within the cortex. 1) All of V1 projects to V2, but only dorsal V1, representing inferior visual fields, projects to V3 (Van Essen et al., J. Physiol. 277, 1978). 3) V2 is 10-15 mm wide along much of its extent, except near the representations of the far periphery (medially) and the fovea (laterally). In the region of far peripheral representation, near the anterior tip of the calcarine sulcus, there is a complete gap of about 1 cm between dorsal and ventral subdivisions of V2; the intervening strip of cortex (the prostriate area of Sanides) is characterized by its distinctive architecture and by an absence of interhemispheric connections along the border with V4. Laterally, near the foveal representation, there is a distinct transition in myeloarchitecture at the border between V2 and V4. The minimum width of V2 in this region varies from 1 to 2 mm (with a possibility of a complete gap) in two other cases we have examined. 4) The detailed organization of inputs to V2 was examined by marking injections made with iontophoretic techniques. Marked injections in V1 (1 mm in extent) within V1. As reported by others, dorsal V1 projects to dorsal V2, ventral V1 to ventral V2, and the horizontal meridional representation of V1 to both subdivisions of V2. Within each subdivision, however, the pattern of inputs from V1 is variable. In some cases the projection terminates as a single patch 2-4 mm across, but in other cases the area is divided into two patches, each 2-3 mm across and separated by a gap of 0.5-1 mm [cf. Weller et al., Arvo Abstr., 1979]. Moreover, the inputs to individual patches appear to be organized in a manner which may reflect a specific pattern of organization at a sub-millimeter level. Altogether, these experiments indicate several significant differences in the organization and connections of V2, V3 and V4 compared to previous descriptions.

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Although the projections of lateral striate cortex, the part representing central vision, have been extensively studied in the rhesus monkey, there are only a few reports of projections of posterior and medial striate cortex, parts representing peripheral and far peripheral vision, respectively. We therefore investigated the cortical efferents from the entire area 17, the largest projection fields are located in area 17 with little or no invasion of area 18; their brains were processed for terminal degeneration by the Fink-Heimer procedure. In a second series, selected striate sites were injected with tritiated amino acids, and the brains processed for autoradiography; representations of the injection sites ranged from 4° to 25° from fixation in either the upper or lower visual field. The results indicate that striate cortex projects to at least three separate and topographically organized visual areas within prestriate cortex.

The largest projection field is a circumstriate cortical belt which corresponds remarkably closely to area OB of von Bonin and Bailey. It completely surrounds area 17 along the 17-18 border (except at the representation of fixation). Within this visual area, the representations of the upper and lower visual fields are entirely separate. Progression from central to far peripheral vision is represented: a) in the lower field, by a progression into the posterior bank and depth of the lunate sulcus, medial to the posterior end of the suprasylvian sulcus; b) in the upper field, by a progression into the inferior occipitotemporal and collateral sulci, and then rostrally along the upper lip of the calcarine fissure; c) the calcarine fissure; and d) by a progression into the posterior and medial striate cortex, parts representing far peripheral vision.
mirror images. Therefore in conjunction with the prominent upper
animals.

place of response was reversed. Thus it appears that attention to
place of response, often at the top or bottom of the stimuli. To
monkeys find LR easier to learn than UD.

but reversed projection through the interhemispheric commissures.

patterns. With the peg aligned with the axis of symmetry of the
stimuli, LR were learned more easily than UD, as pre­

In support of this, optic chiasm sectioned monkeys taught to dis­
mistaken for left and right in normal animals.

For example, animals could code stimulus orientation relative to
asymmetries in the world, can occur during discrimination of
response was half the magnitude of that associated with masking.

A similar experiment with normal monkeys showed that they, too,
reversed their performance on LR postfixed that extracellular accumulations of
place of response was reversed. Thus it appears that attention to
cues near the place of response, or coding orientation relative to
in a WGTA equipped with a vertical panel containing planimetric
interpretation ascribes the difficulty to confusion between the
pretectal connectivity. First, the pretectum does not project
physiological outflow involved in the pupillary light reflex. The
pretectum does, however, project upon visually associated cortical regions.

POTASSIUM RELEASE AND CURRENT FLOW IN THE FROG RETINA: A TEST OF
THE MÜLLER CELL HYPOTHESIS OF ELECTRORETINOGRAM (ERG) B-WAVE
GENERATION. David A. Vogel and Daniel G. Green. University of
Michigan.

The electroretinogram is an electrical potential that develops
across the retina in response to a flash of light. One of the
components of the pigment epithelium ERG, the b-wave, has been used by many investiga­
tors as a measure of responsiveness of the retina. Despite its
common use, the exact origin of the b-wave remains unknown.

It has been postulated that extracellular accumulations of
potassium ions may act to locally depolarize the membrane of the
 Müller cell. This depolarization should then be the site of the
signal for the extracellular current flow which causes the b-wave.

A strong test of the Müller model is to compare the distributions
and the time courses of development of the known K⁺ increase and the
b-wave current b wave-wave current b-wave current curve.

The light adapted eyecup preparation from Rana pipiens was used
for this experiment. Local ERG’s and K⁺ concentration were measured simultaneously using
a double labeled technique. The extracellular sources and sinks were calculated by computer.

The prediction from the Müller cell model is that the ERG
current sinks and sources osmotic activity increase.

The above findings are a direct contradiction to the results
predicted by the Müller cell hypothesis. Therefore, if the
Müller cell plays any role in generation, it must be in
conjunction with other retinal cells.

The left optic nerve of a black, 111-day-old, male
C57BL/6J-c2J mouse was fixed (aldehyde), lead citrate) on a Formvar-coated slot
grid. A photomontage of the section was made from se­
ual electron micrographs. Axons were counted in 25
sample areas (each representing l0µm X l0µm, magnifi­
cation = 3,750), and then evenly distributed across the entire
section.

The section’s shape was round-to-oval. Its longest
diameter was 400µm, and its shortest was 240µm. The
section’s area was about 100,000µm². Thus, the
sample area of 2,500µm² (25 samples, 100µm² each).
represented about 4% of the total area of the section. The
number of axons counted in this small sample was 2,650.
This leads to an estimated axon count on the order of
100,000 of which about 10% were axons of the retinal

The present data suggest that the earlier, higher estimates and the
later, lower estimates.

The left optic nerve of a black, 111-day-old, male
C57BL/6J-c2J mouse was fixed (aldehyde), stained (cinnam­
um tetroxide), dehydrated (ethanol) and embedded (Epon
812). A thin whole-nerve cross section was stained (u­
ralyl acetate, lead citrate) on a Formvar-coated slot
grid. A photomontage of the section was made from se­
ual electron micrographs. Axons were counted in 25
sample areas (each representing 10µm X 10µm, magnifi­
cation = 3,750), and then evenly distributed across the entire
section.

The section’s shape was round-to-oval. Its longest
diameter was 400µm, and its shortest was 240µm. The
section’s area was about 100,000µm². Thus, the
total sample area of 2,500µm² (25 samples, 100µm² each).
represented about 4% of the total area of the section. The
number of axons counted in this small sample was 2,650.
This leads to an estimated axon count on the order of
100,000 of which about 10% were axons of the retinal

The present data suggest that the earlier, higher estimates and the
density were more accurate
than the recent, lower estimates. Further, the present
estimates of axon count and density were esti­
more accurate than the recent, lower estimates. Further, the present
densities, though
estimates of axon count and density were more accurate
than the recent, lower estimates. Further, the present
densities, though
estimates of axon count and density were more accurate
than the recent, lower estimates. Further, the present
densities, though
estimates of axon count and density were more accurate
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**DISSECTION OF A NEURAL CIRCADIAN OSCILLATOR SYSTEM IN THE EYE OF APHISIS WITH X-RAYS.** John C. Woulfe* and Felix Brumberg. (SPON: B. Peretz). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The rhythm of compound action potentials (CAPs) from the isolated eye of Aphisidae has been shown to exhibit all the properties of a classical circadian oscillator. The number, location, and mechanism of the oscillator(s) are not yet known. We have used X-rays to disrupt the oscillator function of the eye from its other functions. Irradiation of the eye with 50 kvp X-rays at a dose of 1 krad will stop the expression of the circadian oscillation of CAPs. However, the size and shape of the CAP, the CAP average rate and bursting rhythm, and the response to light are not changed at this dose rate. These results indicate that the circadian oscillator mechanism is unlikely to be associated with the actual generation of the CAPs, the bursting pattern, or the light response mechanisms. Irradiation at lower doses (e.g., 16 krad) allows a circadian rhythm of smaller amplitude to be expressed than in the unirradiated control eye. This result seems to imply that there are probably a number of oscillators in the eye. At large doses all oscillators are inactive but at lower doses some oscillators are active so a rhythm of smaller amplitude is observed. Results of irradiating parts of the eye by using lead shields reveal that irradiation of the anterior (corneal) part of the eye decreases the amplitude of the rhythm slightly while irradiation of the posterior (optic nerve) part decreases the rhythm greatly (perhaps totally) implying the largest concentration of oscillators is in the posterior part of the eye. Of the 82 eyes irradiated with doses between 2 and 500 krad, none showed any sign of an ultradian rhythm previously reported in eyes with the front part cut away (Jacklet and Gronerino, 1971). This X-ray "dissecting" technique appears to be superior to the technique of cutting away parts of the eye as far as separating oscillator function from the eye from its other functions. These X-ray results are similar to the Actioninon D effects in blocking the circadian rhythm (Bisman and Brumberg, 1975) and imply that RNA transcription may be important in generating the CR. [This work was supported by NIH grant NS-07011 to F.B.]

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Visual input to the optic tectum of the larval Tiger salamander, Ambystoma tigrinum, is being studied using field potential and unit, extracellular recording from posttemporal tectal neurons. In response to optic nerve shock, surface-negative field potential waves are recorded which reverse polarity deeper in the tectum. These waves correspond to layers of coarse terminal degeneration following enucleation (Rice and Jakoby, Brain Res. Proc., 5: 77, 1972). Current sources are located adjacent to the sinks. Since very few cell bodies are located in this region of the tectum, these results suggest that the tectal reaction may be a result of dendro-dendritic synapses onto the distal dendrites of deeper-lying tectal cells. Visual stimulation using flashed spots of light also reveals a retinal input. Input from the superficial layers of the optic tectal neuropil. ON-OFF and OFF unit responses are localized to the same depth as the two deeper current sinks evoked by optic nerve shock. On-going experiments are attempting to associate visual afferent units with the most superficial and slowest current sink. These responses are also being correlated with posttemporal synaptic potentials elicited by similar stimuli and recorded intracellularly in deep tectal cells that are being recovered after dye injection. The presence in most cells of inhibitory posttemporal potentials of a transient ON-OFF nature and large visual receptive field indicate that the ON-OFF retinal afferents participate, either directly or indirectly, in a global tectal inhibitory system.

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**THE GABA SYSTEM IN GOLDFISH RETINA: A COMPARATIVE ANALYSIS WITH N-GLUTAMATE AND N-MUSCIMOL.** Stephen Yarull* and R. Breche (SPON: P. Witkowsky). Department of Biology and Psychiatry, SUNY, Stony Brook, NY 11794.

Mammalian, a GABA analogue with a reported low affinity for GABA uptake sites, was used initially to study synaptic binding to GABA receptors in goldfish retina. In retinal homogenates N-muscimol (NM) shows saturable binding with a Kd of 10^-8 M, a receptor concentration of 160 fmoles/mg-protein and is blocked by GABA at an IC50 of 4x10^-7 M. Localization of labeling by autoradiography shows heavy label over 4 distinct lamina in the inner plexiform layer (IPL) and over a population of amacrine cell bodies and lightly over cone horizontal cell bodies (HC). GABA (1mM) abolished labeling over HC's and the proximal IPL but had little inhibitory effect on amacrine cells and the distal IPL. Since the NM labeling pattern appeared partially due to uptake and was differentially inhibited by GABA, we performed parallel incubations in NM and N-GABA (NGA) under a variety of conditions designed to block uptake mechanisms. The normal GABA pattern differs from that of NM and includes intense label over cone HC bodies and axon terminals (AT), some amacrine cells in the proximal IPL rather than throughout. Incubation in the presence of 1mM muscimol does not inhibit GABA uptake. The specific GABA uptake blocker GABA (2.5mM) slightly suppresses NM labeling in the IPL and strongly inhibits GABA labeling in the proximal IPL while sparing the remaining patterns. Incubation in Na+-free Ringers or in the presence of chloroquine (0.1mM) eliminates somal label of NM leaving a reduced but obvious pattern in the IPL. Oubain similarly affects the GABA pattern, while in Na+-free Ringers, consider- ably enhanced GABA uptake is seen in amacrine cell bodies and the distal IPL (i.e., resembles the GABA block on NM). From these results we conclude that the binding of NM at GABA synaptic receptors can be measured biochemically in the retina. Localization of NM binding by ARB is severely limited by uptake contamination, although with proper controls NM binding is seen in the IPL but not in the OPL. There are at least two "species" of GABA uptake sites: 1) on somatic and proximal IPL, 2. amacrine cells and distal IPL, and 3, NA. Finally there is some, as yet undetermined, relation between NM labeling and Na+-free Ringers uptake sites. These results suggest that GABA uptake is unique to goldfish but have been duplicated to a large extent in chick retina.

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We studied the pupil response by measuring the change in the deep postonucal (D) peak of the transepithelial current sink which reverse polarity deeper in the tectum. Laminar currents in deep tectal cells that are being recovered after optic nerve shock. The action spectrum for a criterion reflection increase is the same as the electrophysiological sensitivity of the predominant photoreceptor type, R1-6; the curve is like one obtained by measures of antidromic transmission in the deep pseudopupil of the white-glacoc (w) mutant (Franceschini, iibd.).

Bright short wavelength irradiation induces a post-stimulus Prolonged Pupillary Response (PPR) which is very long-lived (several hours). This is like the Prolonged Depolarizing Afterpotential (PDA) induced after intense blue light substantially converting the 480 nm absorbing rhodopsin to its 590 nm absorbing stable metarhodopsin. The PPR is extreme at λ<530 nm and is absent at λ>550 nm. The PPR becomes noticeable after delivering I= 8×1013 quanta/cm2 and saturates around I=2×1015 quanta/cm2 (λ=441 nm). Both PPR induction and suppression are determined by the total number of quanta delivered. Thus the PPR is an integrating phenomenon in the D peak. The PPR is eliminated by vitamin A deprivation as is the PDA.

The mutant transient receptor potential (trp) discovered by Costas and Nirenberg (1985) shows a transient pupillary response upon light adaptation. The full response is reached only after a long dark adaptation time (5-10 min.). This is slightly longer than wild-type sensitivity in the deep PPR recovery in trp (Wink, Biophys. Soc. Proc. 33, 59, 1977). The PPR induced by blue light in trp is only partially closed pupil. In accordance with trp's receptor potential's PDA, Long wavelength light is necessary to renew the pupil's excitability (after a dark adaptation period). The sensitivity of the trp puppil is the same as the wild-type (after long wavelength illumination then dark adaptation).

Pupil and receptor potential mechanisms in Drosophila have very similar characteristics. Thus, there has been a critical new window on pupil mechanisms with non-optical visual techniques.